Available online on 15.11.2021 at <http://jddtonline.info>

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Copyright © 2021 The Author(s): This is an open-access article distributed under the terms of the CC BY-NC 4.0 which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited



Open Access Full Text Article



Research Article

GC-MS, FTIR and Antiulcer Screening of Aqueous Seed Extract and Oil of *Nigella sativa* in Wistar Rats

Bruce, S.O.¹, Nwafor, O.I.¹, Omoirri, M.A.², Adione, N.M.³, Onyeka, I.P.¹, Ezeoru, V.C.⁴

¹ Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

² Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences. Federal University of Oye-Ekiti, Ekiti state.

³ Department Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

⁴ Department of Laboratory Science, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

Article Info:



Article History:

Received 13 September 2021
Reviewed 18 October 2021
Accepted 25 October 2021
Published 15 November 2021

Cite this article as:

Bruce SO, Nwafor OI, Omoirri MA, Adione NM, Onyeka IP, Ezeoru VC. GC-MS, FTIR and Antiulcer Screening of Aqueous Seed Extract and Oil of *Nigella sativa* in Wistar Rats, Journal of Drug Delivery and Therapeutics. 2021; 11(6):48-60

DOI: <http://dx.doi.org/10.22270/jddt.v11i6.5036>

*Address for Correspondence:

Bruce, Stella Omokhefe, Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

Abstract

Peptic ulcer disease (PUD) is a sore in the lining of the stomach or duodenal mucosa. This study was aimed at evaluating the antiulcer activity of the aqueous extract of *Nigella sativa* (black seed) and its constituents. The pharmacognostic properties of the dry seeds were determined. The seeds were extracted using two methods digestion and hydro-distillation. The acute toxicity, phytochemical constituents and the antiulcer evaluation were performed on ethanol-induced ulcer in wistar rats using a standard method. FTIR and GC-MS analysis of the aqueous seed extract was also determined using standard methods. *Nigella sativa* seed shows the presence of starch grains, lignified tissues, tannins, cellulose, protein and oil globules. The aqueous extract has a high safety margin. The phytochemical studies revealed the presence of saponins, flavonoids, alkaloids, tannins, glycosides, fats and oil. The black seed aqueous and oil extract at 500 mg/kg significantly reduced the acidity, total acidity, and ulcer index, and pH of gastric content when compared with the positive control (Famotidine). The FTIR analysis identified the presence of the following functional groups chloro, ether, amine, carboxylic acid, nitriles, methylene, alcohol, while the GC-MS identified five compounds such as glycerin, n-Hexadecanoic acid, 9, 12-octadecadienoic acid-methyl ester, 9, 12-octadecadienoic acid and 9, 12-octadecadienoyl chloride. The pharmacognostic properties can act as a reliable tool for the standardization of the plant part. This study suggests that aqueous and oil extract possess antiulcer properties. Thus the aqueous and oil extract of black seed can be considered as antiulcer medication traditionally.

Keywords: *Nigella sativa*, Black seed, Pharmacognostic, Phytochemical, Acute toxicity (LD₅₀), Antiulcer.

INTRODUCTION

Nigella sativa L, known as black cumin or black seed (family, Ranunculaceae) is used as a spice in Indian and Middle Eastern cuisine. In Asia, Middle East, and Africa, it is used as an herb and oil. Gastrointestinal tract (GIT) is responsible for the absorption of nutrients, digestion of food, and excretion of unabsorbed waste products¹. It is the organ that provides a continuous supply of water, electrolyte, and nutrients to the whole part of the body. Peptic ulcer disease or PUD is a pathological condition of this organ and refers to the painful spot characterized by the presence of an ulcer in any part of GIT which is exposed to acid and pepsin. *Nigella sativa* L ethnomedicinal properties include antimicrobial, antidiabetic, antifertility, antioxytocic, analgesic, anthelmintic, anti-hypertensive, antiulcer, circulatory and immune system support². A Gastro duodenal lesion due to hyperacidity leads

to peptic ulcerations which are erosions of the mucosal epithelial lining of the gastrointestinal tract³. Despite the pathophysiology of peptic ulceration not been completely elucidated, it is known that an imbalance between aggressive factors (acid and pepsin secretion) and cytoprotective factors of the gastric mucus membrane (mucus and bicarbonate secretion) result in gastric ulceration⁴. The most common symptoms of peptic ulcer include epigastric and nocturnal pain which are alleviated by either food intake or antacids; other symptoms include indigestion, nausea, loss of appetite, fatty food intolerance, and heartburn.^{5,6} The aetiology of gastric ulcer involves environmental factors such as alcoholic beverages and non-steroidal anti-inflammatory drugs (NSAIDs) usage, Helicobacter pylori, genetic factors amongst others^{7,8}. The chemical composition of *N. sativa* seeds contains unsaturated fatty acids with terpene alcohols, traces of alkaloids (isochinoline alkaloids and pyrazol alkaloids). In the

essential oil contains thymoquinone, p-cymene, pinene, dithymoquinone and thymohydroquinone². Peptic ulcer of the stomach and duodenum was known in the 1700s, there is a revolution of understanding and management of PUD after the discovery of *Helicobacter pylori*. Several treatments are available for peptic ulcers such as antacids, H₂ blockers, proton-pump inhibitors, antibiotics, and combination therapy. Adverse effects and decreased efficacy over time have limited the use of some anti-ulcer drugs. Reports on clinical evaluation of conventional anti-ulcerogenic drugs showed that there are incidences of relapses, adverse effects and danger of drug interactions during ulcer therapy⁹. The high cost of newly available drugs for peptic ulcer disease is responsible for the persistence, morbidity and mortality of the disease in third world countries due to low per capita income¹⁰. As a result, the search for an ideal antiulcer drug continues and has also been extended to herbs for new and novel molecules that afford better protection and reduction in the incidence of relapse¹⁰. Herbal drugs are used widely even when their biologically active compounds are unknown because of their assumed effectiveness, availability, lesser side-effects and relatively low cost¹¹. The study was aimed to evaluate the GC-MS, FTIR and antiulcer screening of aqueous seed extract and oil of *Nigella sativa* in wistar rats

MATERIALS AND METHODS

Reagents and materials

Ethanol, Ehtylacetate, Butanol, n-Hexane (HD England), Famotidine (CP Pharmaceuticals,UK), Glass column, flasks, beakers, test tubes, measuring cylinders, rotary evaporator, Analytical Weighing Balance (Metler H30, Switzerland), Spectrophotometer (B. Bran Scientific & Instrument Company, England), Water Bath (Techmel & Techmel, Texas, USA), Appendoff tube, plain bottle, and Micropipette (Finnipipette@ Labsystems, Finland).

Sample preparation

The *Nigella sativa* seeds were collected from Roban stores Awka in September, 2020. Samples were authenticated by a taxonomist in the department of botany Mr Okeke Ebele in Nnamdi Azikiwe University Awka, air-dried in a dark room without sunlight, grinded and then they were stored in a cool room (4 °C) for subsequent experiments.

Methods

Extraction Procedures

1. Seed processing

Digestion method was used in extracting the seed. Digestion is an extraction method that involves the use of moderate heat during extraction process. The solvent of extraction is poured into a clean container followed by powdered drug material. The mixture is placed over water bath or in an oven at a temperature about 50 °C¹².

2. Oil extraction

Hydro-distillation method was used in extracting the oil. The air-dried seeds of *Nigella sativa* were grinded right before the extraction process. In order to protect the plant materials from overheating or charring by direct steam, grinded seeds were immersed in distilled water in a round bottom flask on a heater¹³. Essential oil of the seeds was extracted by hydro-distillation Clevenger apparatus method (Fig. 1) at the boiling

range of water and atmospheric pressure. The extraction process was optimized with respect to time, heating power (250, 300, 350 W) and solid (g) to solvent (ml) ratio (1:3, 1:10, 1:12).

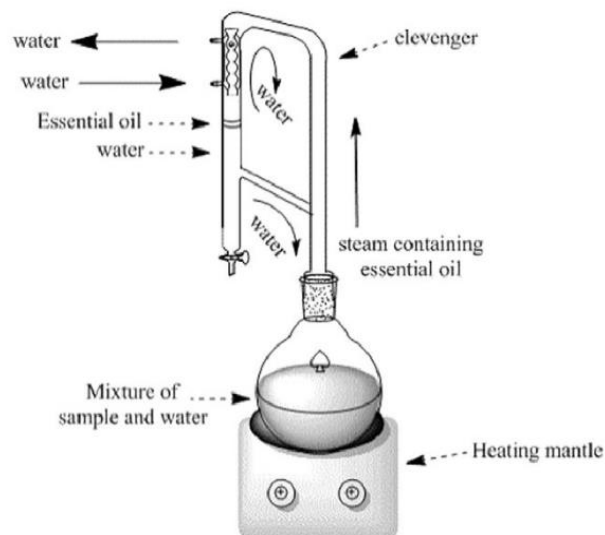


Figure 1: Hydro-distillation Clevenger apparatus system

Extracted essential oil was dried over anhydrous sodium sulfate to remove all the water and then stored it in dark-sealed-vial at 4 °C for further tests. The yield of essential oil was calculated by the following equation:

$$Y = \frac{V \times 100}{W} \quad \text{Equation 1}$$

Where y is the yield of essential oil (%), V is the volume of collected essential oil (ml) and W is the weight of the plant material (g).

Pharmacognostic studies

Microscopic examination

Microscopic studies were carried out by preparing thin sections of leaf. The thin sections were further washed with water, staining was done by clearing in chloral hydrate solution then heat fixed and allowed to cool, then mounted using glycerine. The specimen was gently covered with a cover slip and placed on the stage of the microscope for observation (40x)¹⁴.

Chemomicroscopic examination

Examination of the powder for lignin, starch, mucilage, calcium oxalate crystals, cellulose, fatty oil and protein were carried out using standard techniques¹⁵.

Physicochemical analysis

The parameters which were studied are moisture content, ash values and extractive values^{16, 17}.

Phytochemical analysis

Qualitative phytochemical analysis

The plant crude extracts were tested for the presence Reducing sugar, Hydrogen cyanide, Soluble carbohydrate, Tannins, Alkaloids, Steroids, Terpenoids, Phenol, Flavonoids, Saponins and Glycosides using standard methods¹⁵.

Acute Toxicity

The acute toxicity study of *Nigella sativa* was carried out according to the method employed by Lorke's method¹⁸ but modified, using a total of 21 rats^{19,20}.

Animal husbandry

Thirty Wistar Rats (120–150 g) were obtained from the animal house, Department of Pharmacology and Toxicology, Nnamdi Azikiwe University Awka. They were fed with grower mash (vital feed, grand cereal) and water and kept for 2 weeks to acclimatize with the animal house conditions (a cross-ventilated room with temperature between 25 °C and 32 °C, 12 h light/12 h dark cycle) before the commencement of the study. The research was conducted in accordance with the Nnamdi Azikiwe University Research and Ethical Committee guidelines, the ARRIVE guidelines (reporting of in vivo experiment), and the National Institutes of Health (NIH) guide for the CARE and use of laboratory animals (NIH Publications No. 8023, revised 1978).

Experimental Design:

The thirty (30) Wistar rats were randomly divided into six groups of five rats each and fasted for 18 h before administration of extract. Rats in Group 1 were pretreated with 1 ml/kg Distilled water, the rats in Group 2 were pretreated with 30 mg/kg Famotidine, while those in Groups 3 and 4 were pretreated with aqueous extract of *N. sativa* at 250 mg/kg and 500 mg/kg. Group 5 and 6 were pretreated with Black seed oil at 1 ml and 1.5 ml, 30 mins before the administration of 1 ml 80% ethanol. All the rats were sacrificed 1 h after 80% ethanol administration using ketamine injection, and the stomachs were cut open, the stomach content were deposited in a beaker, the ulcer index was determined. The gastric content was collected in test tube and centrifuged at 3000 rpm for 10 min, Total acidity (m Eq/L), Gastric acid volume (ml) and pH of the supernatant was measured using digital pH meter^{21, 22, 23}.

Histopathology procedure

The stomachs were fixed in 10% neutral buffered formalin, dehydrated in graded series of alcohol, cleared in xylene, and embedded in paraffin wax. The tissues were sectioned at 5 µm with a rotary microtome and stained with hematoxylin and eosin (H and E) and cresyl violet stain²⁴.

Fourier Transform Infrared Spectroscopic (FTIR) Analysis

Buck scientific M530 USA FTIR was used for the analysis. This instrument was equipped with a detector of deuterated

triglycine sulphate and beam splitter of potassium bromide. The software of the Gram A1 was used to obtain the spectra and to manipulate them. An approximately of 1.0g of samples, 0.5ml of nujol was added, they were mixed properly and placed on the salt pallet. During measurement, FTIR spectra was obtained at frequency regions of 4,000-600cm⁻¹ and co-added at 32 scans and at 4cm⁻¹ resolution. FTIR spectra were displayed as transmitter values²⁵.

GC/MS Analysis

The Gas chromatography-mass spectrometry (GC-MS) analyses of *N. sativa* aqueous seed extract was carried out in Agilent Technologies (Wilmington, Delaware, USA) equipment with a column HP-5MS (30 m × 0.25 mm 1D X 0.25). A sample of 1 µL of each extract was injected. For detection, an ionization system with energy of 70 eV was used. The flow rate of carrier gas was constant and it was maintained at 1.1 mL/min. The injection temperature was 250 °C. The warming program of the oven was isothermal for 5 min at 60 °C followed by a warming of 5 °C/min up to 100 °C/min (2 min), and 10 °C/min up to 250 °C/min (5 min). The interpretation of the mass spectra was made using the National Institute of Standard and Technology (NIST) library^{26, 27, 28}.

Statistical analysis

Data obtained from the study were analyzed using Statistical Package for Social Sciences (SPSS-21). Results were presented as mean ± Standard error of mean (SEM) of sample replicates. Raw data were subjected to one way analyses of variance (ANOVA) followed by post hoc turkey's test. p<0.05 were considered to be statistically significant.

RESULTS

Microscopy

Table 1: Microscopy

Parameter	Black seed
Starch grains	Present
Lignified tissues	Present
Calcium oxalates	Absent
Cystolith	Absent
Tannin	Present
Cellulose	Present
Gum/Mucilage	Absent
Protein	Present
Oil globules	Present

Table 2: Chemomicroscopic Examination

TEST REAGENT	OBSERVATION	INFERENCE
Sample + phloroglucinol + Conc Hcl	Red colour observed	Lignin Present
Sample + iodine	Blue colour observed	Starch Present
Sample + Hydrochloric acid	No crystals dissolved	Calcium Oxalate Absent
Sample + Ruthenium red	No colour change	Mucilage Absent
Sample + clor-zinc iodine or N/50 idoine + 66% H2SO4	Blue colour observed	Cellulose Present
Sample + Sudan IV reagent	Pink colour observed	Fatty acid present
Sample + 1% Picric acid and millions reagent	Red colour observed	Protein Present

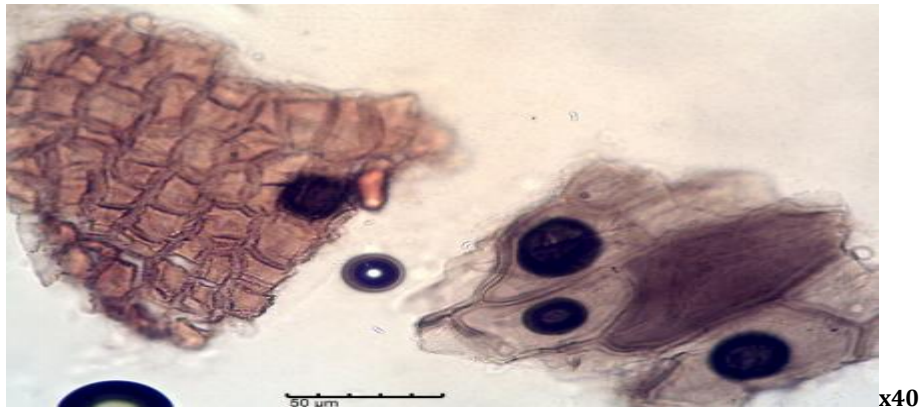
Nigella sativa

Figure 2: Chemomicrograph of the seed powder showing a pack of parenchymatous cells of the cotyledon (lignified) and fraction of the testa showing epidermal cells



Figure 3: Chemomicrograph of the seed powder showing parenchyma cells of the endosperm

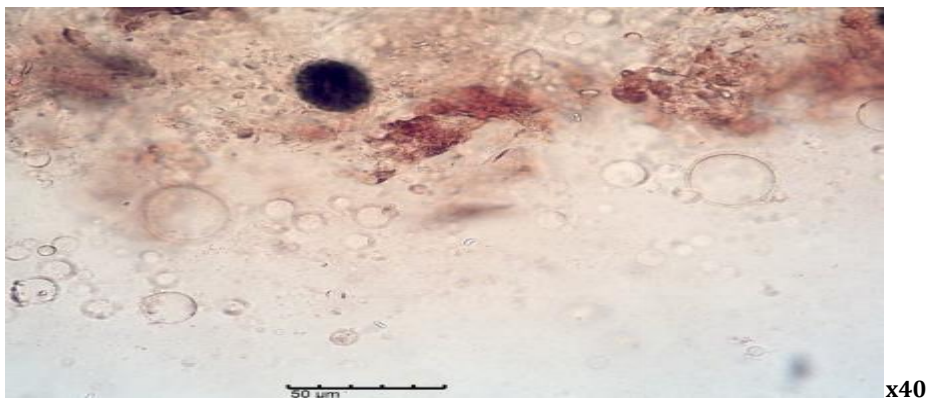


Figure 4: Chemomicrograph of the seed powder showing abundant oil globules/cells and clustered stone cells (lignified)

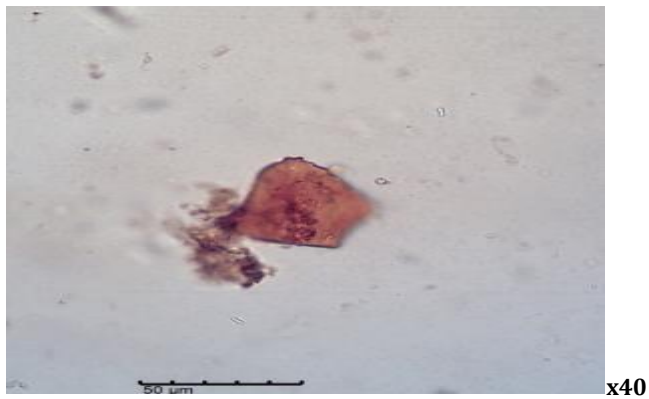


Figure 5: Chemomicrograph of the seed powder showing an isolated lignified stone cell

Proximate analysis

Table 3: Proximate analysis of *Nigella sativa* seed

Parameter	% Composition
Moisture content	5.3
Total ash	4.8
Acid - insoluble ash	1.0
Water - soluble ash	1.3
Acid insoluble ash	1.0
Alcohol - soluble extractive	7.0
Water - soluble extractive	8.5

Values of % composition

Phytochemical Analysis

Table 4: Phytochemical Analysis of *Nigella sativa* seed

Phytochemicals	Crude extract
Saponins	++
Tannins	+
Carbohydrates	+
Reducing Sugars	+
Flavonoids	+++
Alkaloids	+++
Glycosides	+
Steroids	+
Fats and oils	+++
Proteins	+
Acidic compounds	+

(-) = Not Present, (+) = Present in small concentration, (++) = Present in moderately high concentration, (+++) = Present in high concentration.

Acute toxicity

Table 5: Acute toxicity of *Nigella sativa* seed

PHASES	DOSE (mg/kg)	MORTALITY
PHASE 1	10	0/3
	100	0/3
	1000	0/3
PHASE 2	2000	0/3
	3000	0/3
	4000	0/3
	5000	0/3

Table 6: Dose dependent studies of *Nigella sativa* seed extract using ethanol induced ulcer model rat model

Pre-treatment	Post-treatment	Ulcer Index	Total Acidity (m Eq/L)	Gastric Acid Volume (ml)	pH
Distilled water (1 ml/kg)	80% ethanol	13.00 ± 0.54**	120.4 ± 0.40**	7.20 ± 0.09**	2.2 ± 0.10**
Famotidine (30 mg/kg)	80% ethanol	4.66 ± 0.42*	59.1 ± 0.77*	4.18 ± 0.31*	4.5 ± 0.13*
Aqueous extract (250 mg/kg)	80% ethanol	8.68 ± 0.54*	89.09 ± 0.74*	6.07 ± 0.51*	4.7 ± 1.0*
Aqueous extract (500 mg/kg)	80% ethanol	4.05 ± 0.9*	55.6 ± 0.13*	4.45 ± 0.5*	3.67 ± 1.76*
Black seed oil (1 ml)	80% ethanol	8.87 ± 0.4*	80.9 ± 0.33*	6.2 ± 0.45*	3.74 ± 1.86*
Black seed oil (1.5 ml)	80% ethanol	4.62 ± 0.19*	67.1 ± 0.31*	5.00 ± 0.71*	3.54 ± 2.00*

Values are presented as mean ± Standard error of mean (SEM), n=5. Statistical comparisons as follows: significant at $p < 0.01^{**}$ and $p < 0.05^*$ compared to negative control group.

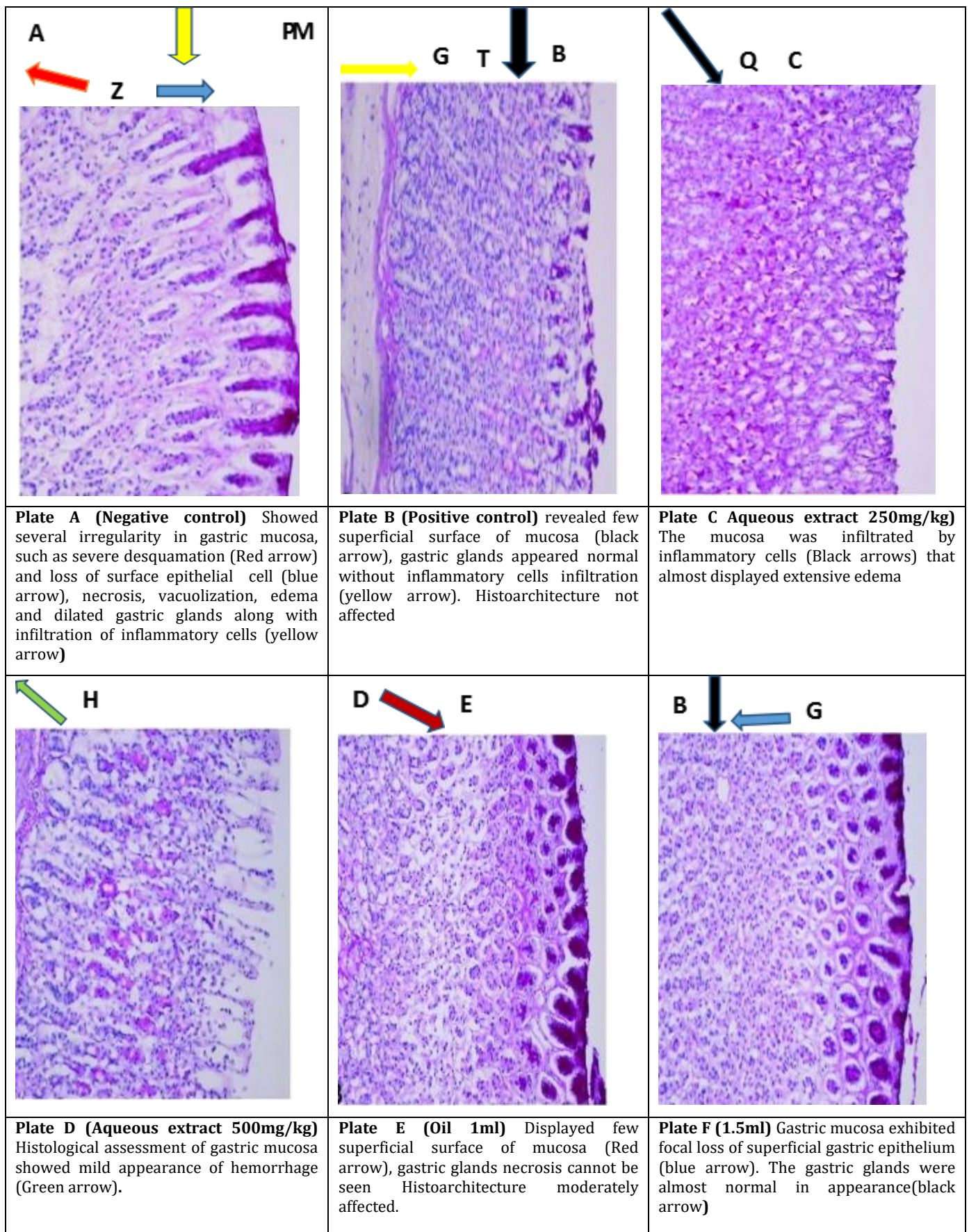
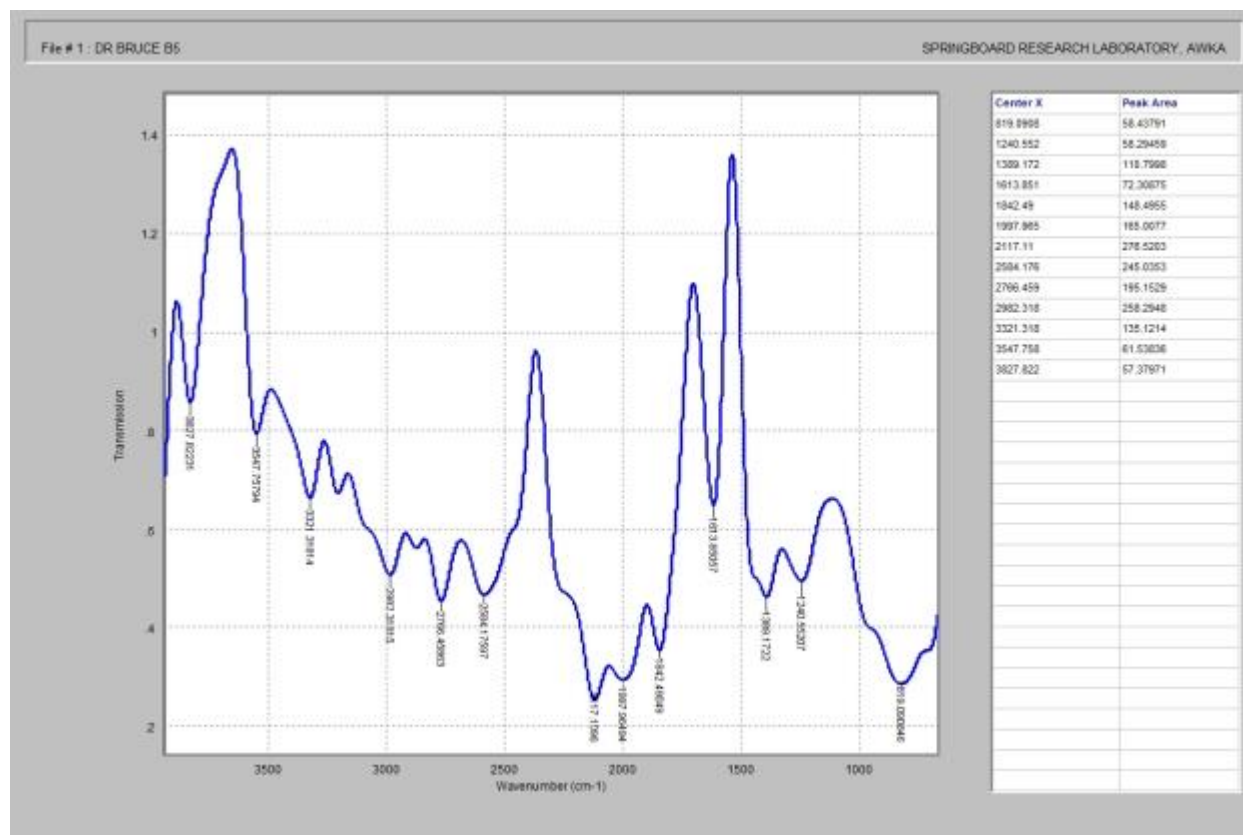


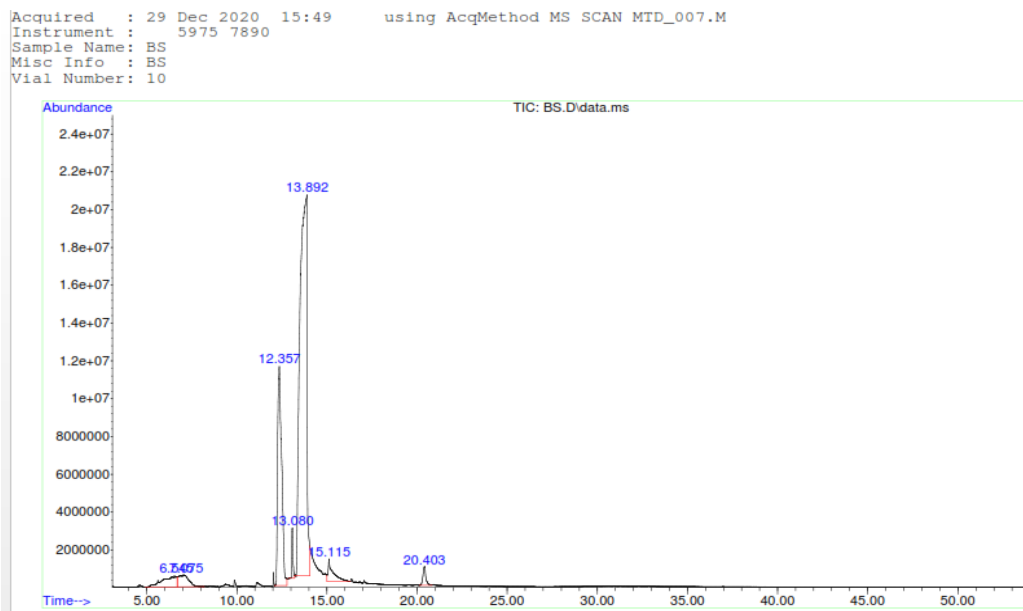
Figure 6: Histology of the isolated stomach of experimental rats

Fourier transform infrared spectroscopy (FTIR)

Figure 7: Fourier transform infrared spectroscopy (FTIR) of *Nigella sativa* seed extractTable 7: Interpretation of FTIR Spectra of *Nigella sativa* seed extract

S/N	Frequency	Functional group	Compounds
1	819.0908	C-Cl	Chloro Cl symmetric stretch
2	1240.552	R-O-R	Ether C-O symmetric stretch
3	1389.172	H ₂ C=CH ₂	Ethene CH symmetric stretch
4	1613.851	RNH ₂	1 ^o amine NH stretch
5	1842.490	R-C=O	Cyclo ester C-O stretch
6	1997.965	R-S-C≡N	Thiocyanate SCN antisymmetric stretch
7	2117.110	RCOOH	Carboxylic acid C-O stretch
8	2584.176	R-C≡N	Nitriles CN antisymmetric stretch
9	2766.459	CH ₂	Methylene CH stretch
10	2982.318	R-S-C≡N	Thiocyanate SCN antisymmetric stretch
11	3321.318	RCH ₂ OH	1 ^o alcohol OH stretch
12	3547.758	R ₃ CHOH	3 ^o alcohol OH stretch
13	3827.822	R ₃ CHOH	3 ^o alcohol OH stretch

Gas Chromatography Mass Spectroscopy (GC-MS)

Figure 8: Gas Chromatography Mass Spectroscopy (GC-MS) chromatogram of *Nigella sativa* seed extractTable 8: Interpretation of Gas Chromatography Mass Spectroscopy (GC-MS) of *Nigella sativa* seed extract

PEAK	RETENTION TIME (RT)	AREA	LIBRARY (NIST)	FORMULAR	BIOLOGICAL ACTIVITIES
1	6.545	3.71	Glycerin	$C_3H_8O_3$ 	Laxative, Antibacterial ²⁷ .
2	7.075	2.99	Glycerin	$C_3H_8O_3$ 	Laxative, Antibacterial ²⁷ .
3	12.357	21.76	n-Hexadecanoic acid	$C_{16}H_{32}O_2$ 	Antioxidant, Antiulcer, Antiandrogenic, flavour, Pesticide, 5-Alpha reductase inhibitor, Hypocholesterolemic, Hemolytic, Nematicide, Lubricant ^{27,28} .
4	13.080	1.48	9, 12-Octadecadienoic acid-methyl ester	$C_{19}H_{34}O_2$ 	Antiacne, antiulcer, 5-Alpha reductase inhibitor, Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge ^{27,28} .
5	13.892	66.06	9, 12-Octadecadienoic acid	$C_{18}H_{32}O_2$ 	Antiacne, antiulcer, 5-Alpha reductase inhibitor, Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge ^{27,28} .
6	15.115	2.67	9, 12-Octadecadienoic acid	$C_{18}H_{32}O_2$ 	Antiacne, antiulcer, 5-Alpha reductase inhibitor, Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge ^{27,28} .
7	20.403 20.409	1.33	9, 12-Octadecadienoyl chloride	$C_{18}H_{31}ClO$ 	Antiacne, antiulcer, 5-Alpha reductase inhibitor, Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge ^{27,28} .

DISCUSSION

Gastric ulcer is due to an imbalance between protective and aggressive factors in the stomach, which is caused by the infection of *Helicobacter pylori*²⁹, non-steroid anti-inflammatory drugs, stress condition, and alcohol consumption^{30, 31}. Microscopic and pharmacognostic standardization will help in the identification and authentication of the genuine plant materials, the presence of starch grains, Lignified tissues, cellulose, proteins, and oil globules, as shown in tables 1 and 2. A previous result of the macroscopic characteristics of *N. sativa* seed includes epidermal cells are irregular in shape, trichomes are nonglandular, and stomata are actinocytic with a broad base. Some previous studies also show similar results³². The leaf epidermal features which include types of stomata, epidermal cells, and hairs are significant tools in delimiting the taxonomy in many plants^{33, 34, 35, 36}. Ahmad et al³⁷ reported variations in the pattern of the epidermal cells that can be used as an important microscopic tool to identify many closely related species. The study of trichomes has been found useful by many researchers^{38, 39}.

The proximate composition of *Nigella sativa* seed extract contains moisture content 5.3 %, total ash 4.8 %, acid insoluble ash 1.0 %, water-soluble ash 1.3 %, acid insoluble ash 1%, water-soluble extractive value 8.5 %, and alcohol soluble extractive value 7.0 % as shown in table 3. In this study, the increased value of water-soluble extract indicates that *Nigella sativa* seed extract dissolves more in water than alcohol and also should extract more secondary metabolites. Fluorescence analysis is also required to evaluate the purity of plant crude⁴⁰. The crude drug moisture content should not be greater than 14% w/w⁴¹. The growth of microorganism (yeast and fungi) is enhanced by high moisture content of crude drugs, causing the breakdown of crucial bioactive compounds. The high value of ash is due to contamination and the presence of impurities⁴². Pharmacognostic and physicochemical studies of the plant parts act as a reliable tool for detecting adulteration and plant identification^{43, 44, 45}.

The phytochemical screening of *Nigella sativa* aqueous seed extract showed the presence of saponins, alkaloids, flavanoids, tannins, glycosides, carbohydrates, and reducing sugar. Saponins, flavonoids, alkaloids, fats and oils are present in huge amounts, as shown in table 4. Previous studies of the phytochemical screening of the *P. nitida* seed extract indicate the presence of alkaloids, saponins, flavonoids, cardiac glycoside, terpenoids, protein, and carbohydrates. Some of the phytochemical compounds detected such as glycoside, saponins, tannins, flavonoids, terpenoids, and alkaloids have been reported to have antimicrobial activity^{42, 46}. It is significant to note that alkaloids contribute to plant species fitness for survival. Some alkaloids and saponins have been found to possess antimicrobial activity⁴⁷. Flavonoids have been shown to have antibacterial, anti-inflammatory, antiallergic, antiviral, antineoplastic, antiulcer activity^{48, 49}.

The result of the acute toxicity after 24 hours of administration of the various doses (10, 100 and 1000 mg/kg body weight) of the aqueous extract of *Nigella sativa* in the first phase and the second phase, three-dose ranges were also used 2000, 3000, 4000 and 5000 mg/kg body weight and there was no death after 24 hours. Therefore, the LD₅₀ was taken to be above 5000 mg/kg practically did not cause any death nor any sign of toxicity observed. Okoye et al⁵⁰ carried

out the LD₅₀ of combined aqueous extracts of *Ocimum gratissimum* and *Anacardium occidentale* (1:1) at a dose of 5000 mg/kg orally which practically did not cause any death nor any sign of toxicity observed.

High concentrations of ethanol induce vascular endothelium injury of the gastric mucosa, which become edematous, and congestive, present point and scattered bleeding lesions, focal hemorrhage, necrosis, and giant deep ulcers were visible. The aqueous extract (250 mg/kg and 500 mg/kg) and oil extract (1 ml and 1.5 ml) showed a significant reduction ($p < 0.05$) in ulcer index, total acidity, Gastric acid volume, and pH of gastric secretion, when compared with the negative control Distilled water (1 ml/kg) as shown in Table 6. The effect of aqueous and oil extracts are dose-dependent. The rats pretreated with *N. sativa* aqueous extract (500 mg/kg and 250 mg/kg) and oil extract (1.5 ml/kg and 1 ml/kg) showed a significantly reduction in Ulcer index (4.05 ± 0.9 , 8.68 ± 0.54 and 4.62 ± 0.19 , 8.87 ± 0.4), than those of rats pretreated with the negative control Distilled water (1 ml/kg) (13.00 ± 0.54) at $p < 0.05$. The maximum effect was observed at 500mg/kg of the aqueous extract with ulcer index of (4.05 ± 0.9) and 1.5ml of the oil gave an ulcer index of (4.62 ± 0.19), as compared with the negative control Distilled water (1 ml/kg) with ulcer index of 13.00 ± 0.54 is an indication that the extract can protect the stomach lining from ethanol-induced mucosal damage.

The rats pretreated with *N. sativa* aqueous extract (500 mg/kg and 250 mg/kg) and oil extract (1.5 ml/kg and 1 ml/kg) showed a significantly reduction in total acidity (55.6 ± 0.13 , 89.09 ± 0.74 and 67.1 ± 0.31 , 80.97 ± 0.33), than those of rats pretreated with the negative control Distilled water (1 ml/kg) (120.4 ± 0.40) at $p < 0.05$. The maximum effect was observed at 500mg/kg of the aqueous extract with total acidity of (55.6 ± 0.13) and 1.5ml of the oil gave total acidity of (67.1 ± 0.31), as compared with negative control Distilled water (1 ml/kg) with a total acidity (120.4 ± 0.40). The rats pretreated with *N. sativa* aqueous extract (500 mg/kg and 250 mg/kg) and oil extract (1.5 ml/kg and 1 ml/kg) showed a significantly reduction in Gastric acid volume (4.45 ± 0.5 , 6.07 ± 0.51 and 5.00 ± 0.71 , 6.20 ± 0.45), than those of rats pretreated with the negative control Distilled water (1 ml/kg) (7.20 ± 0.09) at $p < 0.05$. The maximum effect was observed at 500mg/kg of the aqueous extract with the Gastric acid volume (4.45 ± 0.5) and 1.5ml of the oil with the gastric acid volume (5.00 ± 0.71), as compared with the negative control Distilled water (1 ml/kg) with the acid volume (4.18 ± 0.31). Finally, the rats pretreated with *N. sativa* aqueous extract (500 mg/kg and 250 mg/kg) and oil extract (1.5 ml/kg and 1 ml/kg) showed a significantly reduction in the pH of gastric secretion (3.67 ± 1.76 , 4.7 ± 1.0 and 3.54 ± 2.00 , 3.74 ± 1.86), than those of rats pretreated with the negative control Distilled water (1 ml/kg) (2.20 ± 0.10) at $p < 0.05$. The pH of gastric secretion of 500mg/kg of the aqueous extract (3.67 ± 1.76) and 1.5 ml of the oil (3.54 ± 2.00) has the maximum effect, as compared with the negative control Distilled water (1 ml/kg) (2.20 ± 0.10).

Ethanol induces gastric mucosal damage by promoting disturbances of mucosal microcirculation, ischemia, endothelin release, degranulation of mast cells, inhibition of prostaglandins and decrease of gastric mucus production^{51, 52}. Ethanol rapidly penetrates the gastric mucosa causing injury characterized by membrane damage, erosive hemorrhagic lesions with diffuse coagulative cell necrosis, cell exfoliation, multiple superficial erosions, marked vascular congestion and ulcer formation^{53, 54, 55}.

Histological examination of the stomach of rats pretreated with negative control showed irregularity in gastric mucosa, such as severe desquamation (Red arrow) and loss of surface epithelial cell (blue arrow), necrosis, vacuolization, edema and dilated gastric glands along with infiltration of inflammatory cells (yellow arrow). The stomach of rats pretreated with the positive control (Famotide) revealed a few superficial surfaces of mucosa (black arrow), gastric glands appeared normal without inflammatory cells infiltration (yellow arrow). Histoarchitecture not affected. The stomach of rats pretreated with *N. sativa* aqueous extract (250 mg/kg) showed the mucosa was infiltrated by inflammatory cells (Black arrows) that almost displayed extensive edema. The stomach of rats pretreated with *N. sativa* aqueous extract (500 mg/kg) showed the mild appearance of hemorrhage (Green arrow). The stomach of rats pretreated with *N. sativa* oil extract (1 ml) displayed few superficial surfaces of mucosa (Red arrow), gastric glands necrosis cannot be seen histoarchitecture moderately affected. The stomach of rats pretreated with *N. sativa* oil extract (1.5 ml) showed that gastric mucosa exhibited focal loss of superficial gastric epithelium (blue arrow). The gastric glands were almost normal in appearance (black arrow). The healing process of gastric ulcer including several processes in gaster mucous e.g. congestive, hemorrhagic, edema, necrosis, inflammation, erosion, ulceration and dysplastic change⁵⁶. Evaluation for the healing process in the clinical setting was based on visual endoscopy, but this study was based on microscopic evaluation and gastric ulcer determination. Microscopic evaluation showed gastric glands dilatation, increase of connective tissue, increase of micro vascularization, and recovery of a sensory nerve. It could be the basis for evaluating the quality of the healing process of gastric ulcers^{57,58}.

Fourier Transformed Infrared (FTIR) technique is an important tool used to identify the characteristic functional groups, which are instrumental in the determination of functional groups and organic compounds inherent in any given sample. The peak value 819.0908cm^{-1} was assigned to the C-Cl stretching vibration of the halogeneous compound. The absorbance 1240.552cm^{-1} was assigned to the C-O stretching vibration of the ether compound. The peak value 1389.172cm^{-1} was assigned to the C=C stretching vibration of the ethene compound. The medium band 1613.851cm^{-1} corresponds to the NH stretching vibration of 1° amine compound. The absorption 1842.490cm^{-1} was assigned to the C=O stretching vibration of the cyclic ester compound. The peak value 1997.965cm^{-1} and 2982.318cm^{-1} were both assigned to the SCN stretching vibration of thiocyanate compound respectively. The band 2117.110cm^{-1} was assigned to the C=O anti-symmetric stretching vibration of carboxylic acid whereas the absorbance 2584.176cm^{-1} was assigned to the CN anti-symmetric vibration of nitrile compound. The weak band 2766.459cm^{-1} was assigned to the C-H stretching vibration of methylene compound. The broadband 3321.318cm^{-1} , 3547.758cm^{-1} and 3827.822cm^{-1} were assigned to OH stretching vibration of 1° & 3° alcoholic compounds respectively, which revealed the highest peaks. Other functional groups such as chloro, ether, amine, carboxylic acid, nitriles, and methylene were equally present (table 7). The presence of various functional groups and phytocompounds in *Nigella sativa* confirm that it acts as the most important source of drugs against various ailments. Onyemalu et al ⁵⁹ carried out UV-Visible and FTIR Spectroscopic Analysis of the crude ethanolic extract of *Peuraria phaseoloide* Leaf (Roxb) Benth.

(Fabaceae) and the FTIR analysis revealed the highest peak at 3291.098cm^{-1} , which signifies the presence of the R₂NH 2O amine functional group. Other functional groups such as halogen, ether, ethane, carboxylic acid, carbonyl, nitrile, and alcohol were equally present.

GC-MS analysis is followed by library search of non-polar extract of the seed identified five compounds such as glycerin, n-Hexadecanoic acid, 9, 12-octadecadienoic acid-methyl ester, 9, 12-octadecadienoic acid and 9, 12-octadecadienoyl chloride. The compounds from the GC-MS investigation indicate the biological properties and in turn the pharmaceutical values of the study plant extract.

The GC-MS techniques have been proven to be suitable for the chemical profiling of medicinal plants⁶⁰, these techniques provide a sufficient profile and identification of the compounds analyzed⁶¹.

Glycerin is known as glycerol, it is widely used in FDA-approved wound and burn treatments. It has been identified with retention times 6.545 and 7.075, it has laxative and antibacterial properties²⁷. Glycerol is a simple polyol compound. It is a colorless, odorless, viscous liquid that is sweet-tasting and non-toxic. The glycerol backbone is found in lipids known as glycerides. Glycerol has been detected in a good number of propolis samples from different regions around the world for example in Turkish propolis, Canadian propolis and Brazilian geopropolis^{62, 63, 64}.

n-Hexadecanoic acid (palmitic acid) is a long-chain saturated fatty acid with a 16-carbon backbone commonly found in milk products, meat, and oil. It is the most common saturated fatty acid found in animals, plants and microorganisms. n-Hexadecanoic acid has been identified with a retention time of 12.357, it also has the following properties; nematocide, pesticide, lubricant, anti-androgenic, flavor, hemolytic 5- α reductase inhibitor, antioxidant and hypo-cholesterolemic features⁶⁵.

Among the identified phytochemicals, n-Hexadecanoic acid, 9, 12-octadecadienoic acid-methyl ester, 9, 12-octadecadienoic acid and 9, 12-octadecadienoyl chloride have the property of antioxidant, hypocholesterolemic, nematocide, pesticide, lubricant, antiacne, antiulcer, 5- α reductase inhibitor, antiandrogenic, antiarthritic, and anticoronary activities^{66, 67}. n-Hexadecanoic acid as the common compound in the leaves of *P. stratiotes*, *Peuraria phaseoloides* and *E. crassipes*.

9,12,15- Octadecatrienoic acid, methyl ester, (Z,Z,Z)- is a polyenoic fatty acid compound and it may act as an anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematocide, insectifuge, anti-histaminic, anti-arthritic, anticoronary, anti eczema, anti-acne, 5- α reductase inhibitor and anti-androgenic^{68, 69}.

CONCLUSION

The pharmacognostic properties can act as a reliable tool for the standardization and quality evaluation of the plant part. The anti-ulcerogenic properties exhibited by the plant extracts are due to the presence of secondary metabolites, this study suggests that aqueous and oil extract possesses antiulcer properties and thus the aqueous and oil extract of black seed can be considered as antiulcer medication traditionally.

ACKNOWLEDGMENTS

The authors are thankful to Department of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe University, Awka, Nigeria, for access to scanning electron microscopy (SEM).

CONFLICT OF INTEREST

The authors have no conflict of interest.

REFERENCES

- Singh B, Solanki RK, Status of seed spices research and development in India. *Indian Journal of Agricultural Sciences*, 2015; 85(2):151-6.
- Sharma NK, Ahirwar D, Jhade D, Gupta S, Medicinal and Pharmacological Potential of *Nigella sativa*: A Review. *Ethnobotanical Review*, 2009; 13:946-55.
- Oliveira AP, Santin JR, Lemos M, Klein-Junior LC, Couto AC, Meyre da Silva Bittencourt C, Cechinel-Eliho V, Faloni de Andrade S, Gastroprotective activity of methanol extract and marrubium obtained from leaves of *Marrabimum vulgare* L. (Lamiaceae). *Journal of Pharmacy and Pharmacology*, 2011; 63:1230-1237. <https://doi.org/10.1111/j.2042-7158.2011.01321.x>
- Ramakrishnan K, Salinas RC, Peptic ulcer diseases. *American Family Physician*, 2007; 76:1005-1012.
- Gupta S, Jhade D, Ahirwar D, and Sharma NK, Medicinal and pharmacological potentials of *Nigella sativa*. *Ethnobotanical Review*, 2009; 13:946-55.
- Bruce SO, Onyemaiu VO, Orji CE, Evaluation of the antiulcer activity and GC-MS spectroscopic Analysis of the crude ethanolic extract of *Peuraria Phaseoloide* Leaf (Roxb) Benth. (FABACEAE). *World Journal of Pharmaceutical Research*, 2021; 10(7):39-59.
- Tsukimi H, Nakai S, Itoh K, Amagase S, Okabe S, Involvement of heat shock proteins in the healing of acetic acid-induced gastric ulcers in rats. *Journal of Physiology and Pharmacology*, 2001; 52:391-405.
- Konturek PC, Konturek T, Brzozowski JW, Konturek JW, Pawlik WW, From nerves and hormones to bacteria in the stomach; Nobel prize for achievements in gastrology during last century. *Journal of Physiology and Pharmacology*, 2005; 52:507-530.
- Kommu S, Gowrishankar NL, Shankar M, Suvetha K, Eswaraiiah MC, Evaluation of anti-ulcer activity of methanolic extract of *Balanites aegyptiaca* L. Bark. *International Journal of Phytopharmacology*, 2013; 4:308-10.
- Saha L, Bhatia A, Chakrabarti A, Gastroprotective effect of bezafibrate, a peroxisome proliferator activated receptor α agonist and its mechanism in a rat model of aspirin-induced gastric ulcer. *Advances Digestive Medicine*, 2016; 3:101-10. <https://doi.org/10.1016/j.aidm.2016.04.001>
- Ezekwesili CN, Ghasi S, Adindu CS, Mefor NC, Evaluation of the antiulcer properties of aqueous extract of unripe *Musa paradisiaca* Linn. Peel in wistar rats. *African Journal of Pharmacy and Pharmacology*, 2014; 8(39):1006-1011.
- Ingle KP, Deshmukh AG, Padole DA, Dudhare MS, Moharil MP, Khelurkar VC, Phytochemicals: Extraction methods, identification, and detection of bioactive compounds from plant extracts. *Journal of Pharmacognosy and Phytochemistry*, 2017; 6:32-6.
- Milojevic JSZ, Stojanovic TD, Palic R, Lazic ML, Veljkovic VB, Kinetics of distillation of essential oil from comminuted ripe juniper (*Juniperus communis* L.) berries. *Biochemical Engineering Journal*, 2008; 39:547-553. <https://doi.org/10.1016/j.bej.2007.10.017>
- Khandelwal KR, Practical Pharmacognosy. Pragati Books Pvt. Ltd. 2008; PP 220
- Evans WC, Trease and Evans Pharmacognosy. WB Saunders Ltd. London, 2002; 32, 33, 95 - 99, 512, 547.
- Eleazu C, Eleazu K, Determination of the proximate composition, total carotenoid, reducing sugars and residual cyanide levels of flours of 6 new yellow and white cassava (*Manihot esculenta* Crantz) varieties: *American Journal of Food Technology*, 2012; 7(10):642-649. <https://doi.org/10.3923/ajft.2012.642.649>
- Onyegbule FA, Okoli OG, Bruce SO, "In vivo Evaluation of the Antimalarial Activity of the Aqueous Ethanol Extract of *Monodora myristica* Seed in Albino Mice", *International Journal of Science and Research*, 2019; 8(6):1530 - 1538.
- Lorke DA, New approach to practical acute toxicity test. *Archives of Toxicology*, 1983; 54:279-86. <https://doi.org/10.1007/BF01234480>
- Ihekwereme CP, Bruce SO, Orji CE, Ibe CI, Iloh E, Aqueous extracts of *Ocimum gratissimum* and *Anacardium occidentale* synergises in anti-diarrhoeal property. *International Journal of Modern Pharmaceutical Research*, 2020; 4(4):06-11.
- Onyegbule FA, Bruce SO, Onyekwe ON, Onyealisi OL, Okoye PC, Evaluation of the in vivo antiplasmodial activity of ethanol leaf extract and fractions of *Jatropha gossypifolia* in *Plasmodium berghei* infected mice. *Journal of Medicinal Plant Research*, 2019; 13(11):269-279 <https://doi.org/10.5897/JMPR2019.6766>
- Sabiu S, Garuba T, Sunmonu T, Ajani E, Sulyman A, Nurain I, Balogun A, Indomethacin-induced gastric ulceration in rats: Protective roles of *Spondias mombin* and *Ficus exasperata*. *Toxicology Reports*, 2015; 2:261-7. <https://doi.org/10.1016/j.toxrep.2015.01.002>
- Mshelia HS, Karumi Y, Dibal NI, Therapeutic effect of *Momordica balsamina* leaf extract on ethanol-induced gastric ulcer in Wistar rats. *Annals of Research Hospitals*, 2017; 1:1-5. <https://doi.org/10.21037/arh.2017.04.03>
- Boligon AA, de Freitas RB, de Brum TF, Waczuk EP, Klimaczewski CV, de Ávila DS, Athayde ML, de Freitas Bauermann L, Antiulcerogenic activity of *Scutia buxifolia* on gastric ulcers induced by ethanol in rats. *Acta Pharmaceutical Sinica B*, 2014; 4:358-67. <https://doi.org/10.1016/j.apsb.2014.05.001>
- Farzaei MH, Khazaei M, Abbasabadei Z, Feyzmahdavi M, Mohseni GR, Protective effect of *Tragopogon graminifolius* DC against ethanol induced gastric ulcer. *Iranian Red Crescent Medical Journal*, 2013; 15:813-6. <https://doi.org/10.5812/ircmj.7845>
- Van der Weerd J, Heeren, RMA, Boon JJ, Preparation methods and accessories for the infrared spectroscopic analysis of multi-layer paint. *Studies in Conservation*, 2004; 49:193-210. <https://doi.org/10.1179/sic.2004.49.3.193>
- Onyemaiu VO, Bruce SO, Iloh ES, UV-Visible and FTIR Spectroscopic Analysis of the Crude Ethanolic Extract of *Peuraria Phaseoloide* Leaf (Roxb) Benth. (FABACEAE). *International Journal of Modern Pharmaceutical Research*. 2021; 5(3):148-153.
- Bruce SO, Onyemaiu VO, Orji CE, Evaluation of the antiulcer activity and GC-MS spectroscopic analysis of the crude ethanolic extract of *Peuraria Phaseoloide* Leaf (Roxb) Benth. (FABACEAE). *World Journal of Pharmaceutical Research*, 2021; 10(7): 39-59.
- Gnanavel V, Mary Saral A, GC-MS analysis of petroleum ether and ethanol leaf extracts from *Abrus precatorius* Linn. *International Journal of Pharma and Bio Sciences*, 2013; 4(3):37-44.
- Chai J, Peptic Ulcer Disease. Rijeka, Croatia: InTech. 2011; ISBN: 978-953-307-976-9
- Paguigan ND, Castillo DH, Chichioco-Hernandez CL, Anti-ulcer Activity of Leguminosae Plants. *Argentina Gastroenterology*, 2014; 51(1):64-7. <https://doi.org/10.1590/S0004-28032014000100013>

31. Sultana S, Akram M, Asif HM, Akhtar N, Complementary and Alternative Approaches to Treat Peptic Ulcer. *International Research Journal of Pharmacy*, 2014; 5(5):353-59. <https://doi.org/10.7897/2230-8407.050574>
32. Yousaf Z, Shinwari ZK, Asghar R, Parveen A, Leaf epidermal anatomy of selected *Allium* species, family Alliaceae from Pakistan. *Pakistan Journal of Botany*, 2008; 40(1):77.
33. Naz N, Hameed M, Ashraf M, Ahmad R, Arshad M, Eco-morphic variation for salt tolerance in some grasses from Cholistan Desert, Pakistan. *Pakistan Journal of Botany*, 2009; 41(4):1707- 1714.
34. Hameed M, Ashraf M, Naz N, Al-Qurainy F, Anatomical adaptations of *Cynodon dactylon* (L.) Pers. from the Salt Range Pakistan to salinity stress. I. Root and stem anatomy. *Pakistan Journal of Botany*, 2010; 42(1):279- 289.
35. Riaz A, Younis A, Hameed M, Kiran S, Morphological and biochemical responses of turf grasses to water deficit conditions. *Pakistan Journal of Botany*, 2010; 42(5):3441- 3448.
36. Khan R, Abidin SZU, Mumtaz AS, Jamsheed S, Ullah H, Comparative leaf and pollen micromorphology on some Grasses taxa (Poaceae) distributed in Pakistan. *International Journal of Nature and Life Sciences*, 2017; 1(2):72- 82.
37. Ahmad K, Khan MA, Ahmad M, Shaheen N, Nazir A, Taxonomic diversity in epidermal cells of some sub-tropical plant species. *International Journal of Agricultural and Biology*, 2010; 12(1):115- 118.
38. Adedeji O, Leaf epidermal studies of the species of Emilia Cass. (Senecioneae, Asteraceae) in Nigeria. *Botanica Lithuanica*, 2004; 10(2):121- 133.
39. Adedeji O, Ajuwon OY, Babawale OO, Foliar epidermal studies, organographic distribution and taxonomic importance of trichomes in the family Solanaceae. *International Journal of Botany*, 2007; 3(3):276- 282. <https://doi.org/10.3923/ijb.2007.276.282>
40. Kadam MPV, Yadav KN, Patel AN, Navsare VS, Bhilwade SK, Patil MJ, Phytopharmacopoeial specifications of *Garcinia indica* fruit rinds. *Pharmacognosy Journal*, 2012; 4(31):23- 28. <https://doi.org/10.5530/pj.2012.31.5>
41. Ilanchezhian R, Roshy JC, Acharya RN, Harisha CR, Shukla VJ, Pharmacognostical and physicochemical analysis of bhallataka (*Semecarpus anacardium* Linn.)-fruit. *Pharmacognosy Journal*, 2011; 3(20):9- 16. <https://doi.org/10.5530/pj.2011.20.3>
42. Bruce SO, Onyegbule FA, Ezugwu CO, Pharmacognostic, physicochemical and phytochemical evaluation of the leaves of *Fadogia cienkowskii* Schweinf (Rubiaceae). *Journal of Pharmacognosy and Phytotherapy*, 2019; 11(3):52-60. <https://doi.org/10.5897/JPP2019.0552>
43. Desai D, Chanda S, Pharmacognostic study and physicochemical analysis of leaves of *Terminalia arjuna*. *Pharmacognosy Journal*, 2014; 6(6):15-19. <https://doi.org/10.5530/pj.2014.6.4>
44. Zhao Z, Liang Z, Guo P, Macroscopic identification of Chinese medicinal materials: traditional experiences and modern understanding. *Journal of Ethnopharmacology*, 2011; 131:556- 561. <https://doi.org/10.1016/j.jep.2011.01.018>
45. Raj RSN, Radhamany PM, Pharmacognostic and physicochemical analysis on the leaves of *Brunfelsia americana* L. *Asian Pacific Journal of Tropical Biomedicine*, 2012; 543-546. [https://doi.org/10.1016/S2221-1691\(12\)60178-6](https://doi.org/10.1016/S2221-1691(12)60178-6)
46. Onyegbule FA, Ezenwa CJ, Bruce SO, Umeokoli BO, Standardization, chemical composition and antipyretic evaluation of methanol leaf extract and fractions of *Chrysophyllum albidum* (Sapotaceae). *Tropical Journal of Natural Product Research*, 2020; 4(6):216-222. <https://doi.org/10.26538/tjnpr/v4i6.1>
47. Osborn AE, "Saponins in Cereals", WHO Traditional. *Phytochemistry*, 2003; 62:1-4. [https://doi.org/10.1016/S0031-9422\(02\)00393-X](https://doi.org/10.1016/S0031-9422(02)00393-X)
48. Bruce SO, Onyegbule FA, Biosynthesis of Natural Products, IntechOpen, 2021; DOI: 10.5772/intechopen.97660. <https://www.intechopen.com/onlinefirst/biosynthesis-of-natural-products> <https://doi.org/10.5772/intechopen.97660>
49. Bruce SO, Onyegbule FA, Ihekwereme CP, Evaluation of hepatoprotective and antimicrobial activities of ethanol extracts and fractions of *Picralima nitida* seed and pod. *Journal of Phytomedicine and Therapeutic*, 2016; 1(2):1 - 21.
50. Okoye VO, Bruce SO, Onyegbule FA, Phytochemical screening and pharmacognostic properties of *Peuraria phaseoloides* leaves (roxb) benth. (fabaceae). *International Journal of Public Health, Pharmacy and Pharmacology*, 2020; 5(2):11-24.
51. Awaad AS, Reham ME, Gamal AS, Natural products in treatment of ulcerative colitis and peptic ulcer. *J. Saudi Chem. Soc*, 2013; 17:101-124. <https://doi.org/10.1016/j.jscs.2012.03.002>
52. Arawwawala M, Thabrew I, Handunnetti S, Arambewela L, Anti-inflammatory activity of *Trichosanthes cucumerina* Linn in rats. *Journal of Ethnopharmacology*, 2010; 131:538-543. <https://doi.org/10.1016/j.jep.2010.07.028>
53. Gazzieri D, Trevisani M, Springer J, Harrison S, Cottrell GS, Andre E, Nicoletti P, Gems D, Partridge L, Stress response and aging: "that which does not kill us makes us stronger". *Cell Metabolism*, 2008; 7:200-203. <https://doi.org/10.1016/j.cmet.2008.01.001>
54. Bae, D. K., Park, D., and Lee, S.H. Different antiulcer activities of pantoprazole in stress, alcohol and pylorus ligation-induced ulcer models. *Lab. Animal Res*, 2011; 27(1):47-52. <https://doi.org/10.5625/lar.2011.27.1.47>
55. Baena JM, Lopez C, Hidalgo A, Rame F, Jimenez S, Garcia M, Relationship between alcohol consumption and the success of *Helicobacter pylori* eradication therapy using Omeprazole, Clarithromycin and Amoxicillin for one week. *European Journal of Gastroenterology and Hepatology*, 2002; 14(3):291-296. <https://doi.org/10.1097/00042737-200203000-00014>
56. Janfaza S, Janfaza E, The study of pharmacologic and medicinal valuation of thymoquinone of oil of *Nigella sativa* in the treatment of diseases. *Annals of Biological Research*, 2012; 3(4):1953-7.
57. Tarnawski AS, Ahluwalia A, Molecular Mechanisms of Epithelial Regeneration and Neovascularization during Healing of Gastric and Esophageal Ulcers. *Current Medicinal Chemistry*, 2012; 19(1):16-27. <https://doi.org/10.2174/092986712803414088>
58. Syamsu Rijal, Upik Anderiani Miskad, Djumadi Achmad, Rina Masadah, Dasril Daud, Cahyono Kaelan, Halida Rahawarin, Swandari Paramita, Yadi Yasir, Evaluation of Anti-ulcerogenic Activity in Oil Extract of Jintan Hitam (*Nigella sativa*) Against Ethanol Induced Gastric Ulcer in Mice (*Mus musculus*). *American Journal of Clinical and Experimental Medicine*, 2016; 4(6):179-184 <https://doi.org/10.11648/jajcem.20160406.14>
59. Onyemaiu VO, Bruce SO, Iloh ES, UV-Visible and FTIR Spectroscopic Analysis of The Crude Ethanolic Extract of *Peuraria phaseoloide* Leaf (Roxb) Benth. (FABACEAE). *International Journal of Modern Pharmaceutical Research*, 2021; 5(3):148-153.
60. Sforcin JM, Bankova JM, Propolis: Is there a potential for the development of new drugs? *Journal of Ethnopharmacol*, 2011; 133:253-260. <https://doi.org/10.1016/j.jep.2010.10.032>
61. Gomez-Caravaca AM, Gomez-Romero M, Arraez-Roman D, Segura-Carretero A, Fernandez-Gutierrez A, Advances in the analysis of phenolic compounds in products derived from bees. *Journal of Pharmaceutical and Biomedical Analysis*, 2006; 41:1220-1234. <https://doi.org/10.1016/j.jpba.2006.03.002>

62. Kartal M, Kaya S, Kurucu S, GC-MS analysis of propolis samples from two different regions of Turkey. *Zeitschrift Naturforschung C*, 2002; 57:905-909. <https://doi.org/10.1515/znc-2002-9-1025>
63. Christov R, Trusheva B, Popova M, Bankova V, Bertrand M, Chemical composition of propolis from Canada, its antiradical activity and plant origin. *Natural Product Research*, 2006; 20:531-536. <https://doi.org/10.1080/14786410500056918>
64. Araujo MJAM, Bufalo MC, Bruno JC, Fernandes Jr A, Trusheva B, Bankova V, Sforcin, JM, The chemical composition and pharmacological activities of geopropolis produced by *Melipona fasciculata* Smith in Northeast Brazil. *Journal of Molecular Pathophysiology*, 2015; 4:12-20. <https://doi.org/10.5455/jmp.20150204115607>
65. Komansilan A, Abadi AL, Yanuwidi B, Kaligis DA, Isolation and Identification of Biolarvicide from Soursop (*Annona muricata* Linn) Seeds to Mosquito (*Aedes aegypti*) Larvae. *International Journal of Engineering & Technology IJET-IJENS*, 2012; 12(03): 28-32.
66. Jegadeeswari P, Nishanthini A, Muthukumaraswamy S, Mohan VR, GC-MS analysis of bioactive components of *Aristolochia krysagathra* (Aristolochiaceae). *Journal of Current Chemical and Pharmaceutical Sciences*, 2012; 2:226-236.
67. Uggade A, Anusha B, Characterization and medicinal importance of phytoconstituents of *Carica papaya* from down south Indian region using gas chromatography and mass spectroscopy. *Asian Journal of Pharmaceutical and Clinical Research*, 2013; 6(4):101-106.
68. Wu L, Gao H, Wang X, Ye J, Lu J, Liang Y, Analysis of chemical composition of *Chrysanthemum indicum* flowers by GC/MS and HPTLC. *Journal of Medicinal Plants Research*, 2010; 4(5):421-426.
69. Vohra A, Kaur H, Chemical investigation of medicinal plant *Ajuga bracteosa*. *Journal of Natural Product Plant Resources*, 2011; 1(1):37-45.