Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2021; 10(4): 159-168
Received: 28-05-2021
Accepted: 30-06-2021

Ifeanyi Peter Onyeka
Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria

Charity C Ezea
Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria

Chukwuebuka C Onwuzuligbo
Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria

Cyril Onyeka Ogbue
Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria

Ugonna Chinwe Morikwe
Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria

Corresponding Author:
Ifeanyi Peter Onyeka
Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria

Standardization, anti-oxidants and anti-ulcer potential of *S. nodiflora* and honey

Ifeanyi Peter Onyeka, Charity C Ezea, Chukwuebuka C Onwuzuligbo, Cyril Onyeka Ogbue and Ugonna Chinwe Morikwe

Abstract
Background: *Synedrella nodiflora* is a useful plant in Nigeria, used traditionally for therapeutic treatment of various diseases. Ulcer occurs due to imbalance between the protective and aggressive factors of the stomach caused by inflammation and oxidation as a result of the activities of ulcerogenic factors.
Methods: This study investigated the leaf toxicity profile, phytochemical, and the anti-ulcer effects of a crude leaf extract of *Synedrella nodiflora* combined with honey in rats using the standard laboratory procedures. Anti-ulcer studies were carried out using absolute ethanol and pyloric ligation rat models of ulceration.
Result: The extractive value for *Synedrella nodiflora* was higher with methanol (15.3%) followed by water (13.6%), ethanol (3%), and n-hexane (2.6%). The fiber content and ash value were 5.0 and 6.7 respectively, which implied that the plant material used is of good purity. The acute toxicity showed that the extract caused no death in the mice at 5000 mg/kg, therefore, the LD_{50} is above 5000 mg/kg. No toxicity has been reported of honey in previous studies. The result showed high content of manganese, zinc, calcium and phosphorus in both honey and *Synedrella nodiflora.* These elements play significant roles as antioxidants, they therefore enhanced the activity of the phytomedicine as antioxidant and antiulcer therapy. The result also showed that 100 mg/kg of the crude extract and 1 ml honey had 42.9% and 71.76% inhibition of ulceration, respectively while the same dosage in combination with honey had 74.65% inhibition of ulceration which was significantly higher than the negative control.
Conclusion: The combination of methanol leaf extract of *Synedrella nodiflora* and honey exhibited gastro-protective effect and reduced gastric acid in rats and this may be a proven dimension in treatment of ulceration. This result has proven that honey in combination with crude drugs of *Synedrella nodiflora* is a new dimension in treatment of ulceration and therefore recommends its usage for treatment of ulcer.

Keywords: *Synendrella nodifolra*, ulcer, antioxidant, gastroprotective, minerals

Introduction
Oxidative stress caused by free radicals has a significant negative impact on living cells and have caused numerous disease conditions and sometimes apoptosis. Cellular damage, ulcer, inflammatory diseases, auto-immune diseases, microbial infections and numerous neurodegenerative disorders, are all linked to the activities of free radicals in the human body [1, 2]. Numerous studies have proven that since these disease conditions are mediated by oxidative stress and imbalance between pro-oxidant and antioxidant factors, antioxidants may play a pivotal role in preventing or slowing the progression of these disease conditions. Ulcer a disease condition, occurs as a result of an imbalance between the aggressive factors (acid and pepsin) and mucosal defense factors (mucous, bicarbonate, and prostaglandins) [3] due to oxidative stress. The pathophysiology of ulcer suggest that ulcer is mostly caused by the bacteria *Helicobacter pylori* and persistent use of non-steroidal anti-inflammatory drugs (NSAIDs) [4]. The bacteria and NSAIDs cause ulcers by damaging the mucus of the stomach lining through mechanism of inflammation, thereby exposing the interior of the stomach to acid which irritates the tissue and causes peptic lesion in the stomach [1]. The Economic implication of this oxidative stress and ulcer are huge and therefore calls for the need for continuous search for antioxidant with great antiulcerogenic potential.
There is therefore need for continuous search for effective antioxidant and antiulcerogenic agent from natural origin that are effective, available, and acceptable across various cultures due to its safety, availability and therapeutic efficacy while considering the increasing global public health burdens and costs associated with oxidative stress and ulcer. The Igbo people of South-East Nigeria has a huge culture of use of traditional medicine in the treatment of ulcer and numerous degenerative diseases caused by oxidative stress. *Synedrella nodiflora* (L.) Gaertn is one of the medicinal plants with numerous therapeutic applications across Nigeria.

Synedrella nodiflora is a useful plant in Nigeria used traditionally for therapeutic treatment of various diseases, it is an annual herb of the tropics from the order of Asterales, family of Asteraceae, found spreading as an invasive species throughout the tropical regions of Asia, America and Africa [5]. The leaves are eaten as a vegetable by some livestock and human; and are reported to be rich in flavonoids, alkaloids and tannins [6]. Plant of Asteraceae family consists of herbs which are known to accumulate substantial number of flavonoids and are well reported for anti-inflammatory, antioxidant, antimicrobial, analgesic and antipyretic properties [7]. *Synedrella nodiflora* according to [8] reported to improve fertility rate, litter size and reduced mortality rate from birth to weaning and therefore improves reproduction. The *Synedrella nodiflora* extract of the whole plant was reported to possess anticonvulsant [9], sedative [10], in vitro antioxidant and free radical scavenging [11], antidiarrhoeal agent along with its hypoglycemic potentiality [12] and antinociceptive properties [13]. In Ghana, *S. nodiflora* (L) Gaertn weed is used for the treatment of epilepsy and pain [14]. Also, the leaves of, *S. nodiflora* was reported for amelioration of threatened abortion and as a laxative usually used to feed livestock [15-16]. *S. nodiflora* is also used in Ghana as post-harvest protectants [17].

The people of South-Eastern part of Nigeria traditionally use *S. nodiflora* whole plant for treating cardiac problems, stopping of wound bleeding [14] and for the treatment of ulcer. In Malaysia and Indonesia, the plant is used for as poultice for treating sore legs, headaches, earaches, stomach aches and rheumatism [18].

The anti-ulcer effect of *S. nodiflora* combined with honey is not scientifically reported therefore this study investigated the phytochemicals, proximate, micronutrient, antioxidants and anti-ulcer activities of *S. nodiflora.*

Materials And Methods

Plant Collection

The Fresh leaves of *Synedrella nodiflora* (L.) were collected from its natural habitat at Amawbia Anambra State in June 2019. The plant was properly identified by a plant taxonomist Mr. Felix Nwafor at the Department of Pharmacognosy and Environmental Medicine University of Nigeria Nsuka with voucher number PCG/UNN/0338. The fresh leaves were washed, dried, pulverized and extracted using cold maceration in absolute methanol.

Phytochemical Screening

The qualitative phytochemical analysis was assayed using the method of [19, 20] to determine the presence of secondary metabolites in the *Synedrella nodiflora* (L.) Estimation of the amount of phytochemicals present was assayed using standard laboratory procedures described by [21-24] to determine the amount of phytochemicals present in the leaves. The absorbances were read at 470nm for alkaloids; glycosides at 530 nm; steroids at 540 nm; terpenoids at 538 nm; saponins at 527 nm; flavonoids at 510 nm; tannins at 640 nm and reducing sugar at 510 nm.

Leaf microscopy

The fresh leaf microscopy and powder microscopy was carried out according to the method of [25].

Acute Toxicity Studies

The acute toxicity test to determine the LD_{50} as an index of safety of the extract was done in 2 phases as described by [26] for *Synedrella nodiflora*. Briefly, nine animals (mice) were randomly allocated into 3 groups of 3 rats each. Animals in group 1, 2 and 3 were given 10, 100, and 1000 mg/kg body weights respectively of the extract through the oral route. All the animals survived and were further subjected to acute toxicity test with higher doses in the second trial. In the second trial, 4 animals were randomly allocated to 4 groups of one animal each. Animals in group 1, 2, 3 and 4 were given 1200, 1600, 2900 and 5000 mg/kg body weight, respectively of the extract and were monitored for 2 days (48 hours). Signs of toxicity, mortality and pathological findings were observed. The study followed ethical guideline for investigations using experimental animals established by [27].

Proximate Analysis

The proximate analysis was done by standard method of [28].

Determination of Minerals

The mineral analysis was done by the method of [28] as follows:

The sample was first ashed in the oven at 600 ^{o}C and 2 grams of the samples were analysed for the selected metals (manganese, sodium, Iron phosphate, sulphate and calcium) using standard protocol according to [28] and the mineral content was determined using standard laboratory protocols for determination of minerals at 640, 230, 470, 460, 420 nm respectively using UV-visible absorption spectrometer.

Determination of Vitamins

The vitamin profile was done by the method of [28]

The sample was first ashed in the oven at 600 ^{o}C, and then dissolved with 5ml of 30% HCI and 2 ml of the samples were analysed for the selected vitamins (Vitamin B2, Vitamin B3, Vitamin B6, Vitamin B7, Vitamin B9, Vitamin K and others Vitamins) using standard protocol [28] and the vitamin content was determined using standard laboratory protocols at 510, 560, 540, 460, 420, 520, 379 and 635 nm respectively and values were read with UV-visible absorption spectrometer.

Induction of acute gastric lesion and gastrotherapeutic studies

The anti-ulcer studies of *Synedrella nodiflora* were done by adopting the ethanol-induced ulcer in rat models [29] as described by [30]. Omeprazole was used as standard drug. Initially, the animals were fasted for 36 hours but were allowed free access to water *ad libitum*. They were randomly selected and divided into ten groups of five rats each. Group 1 served as negative control that received only water; group 2 served as positive control that received Omeprazole; group 3 served as untreated control group 4 to 6 received crude extract of *Synedrella nodiflora* at doses of 100, 250 and 500 mg/kg body weight respectively; group 7 to 9 received combination of 1 ml honey and crude extract of *Synedrella nodiflora* at doses of 100, 250 and 500 mg/kg while group 10 received only 1 ml of honey. Thirty minutes after administration of the extract, honey, water and standard drugs, ulceration was induced by gastric instillation of 1 ml of 96% ethanol (5 mL/kg, po), except the normal group that served as normal control. One hour after administration of ethanol, the animals were anesthetized with intraperitoneal administration of ketamine (50 mg/kg). The stomach was dissected and examined for any ulcerative lesions. The number, length and severity of ulceration were scored as follows: 0= normal colored stomach, 0.5= red colored, 1= spot ulcers, 1.5= hemorrhagic streak, 2= ulcers, 3= perforation. Ulcer index

was expressed as Ulcer index= (UN+ US+UP) x 10^{-1}. Where UN means average of number of ulcers per animal, US mean average of severity of ulcer and UP means percentage of animals with ulcer. Also, the Percentage inhibition of ulceration (PIU) was calculated using the formula:

$$PIU = \frac{\text{Ulcer control} - \text{Ulcer treatment}}{\text{Ulcer control}} \times 100$$

Antisecretory studies

The antisecretory assay was carried according to the method of [31]. Excessive gastric acid plays a central role in ulcer induction through pyloric ligation. The ligation of the pyloric region of the stomach causes hyper secretion of the gastric acid which induces the gastric auto-digestion due to accumulation of gastric acid or pepsin [32]. The ligation of the pylorus stimulates pressure receptors initiated by vagovagal reflex in the central mucosa of the stomach. This stimulation of the receptor nerves of the parietal cells leads to hyper-secretion of the gastric acid. The increased level of gastric acid leads to formation of ulceration due to mucosal auto-digestion as noted by [32].

Results

Phytochemicals

The result of the phytochemical screening of *S. nodiflora* is presented in Tables 1 and 2.

Qualitative Phytochemicals Analysis

The result of the qualitative phytochemical analysis is presented in Table 1. The result showed that *S. nodiflora* contained alkaloids, glycosides, flavonoids, tannins, phlobatanins, saponins, proteins, carbohydrates, reducing sugars and anthocyanins while terpenoids, steroids and anthraquinones was absent. Also, honey contained alkaloids, glycosides, flavonoids, tannins, phlobatanins, saponins, proteins, carbohydrates, reducing sugars, anthocyanins, terpenoids, steroids and anthraquinones.

Table 1: Qualitative phyto-constituents in *S. nodiflora* and Honey

SN	Bioactive compound	*S. nodiflora*	Honey
1	Alkaloids	Present	Present
2	Glycosides	Present	Present
3	Flavonoids	Present	Present
4	Tannins	Present	Present
5	Phlobatanins	Present	Present
6	Saponin	Present	Present
7	Steroid	Absent	Present
8	Proteins	Present	Present
9	Terpenoids	Absent	Present
10	Carbohydrates	Present	Present
11	Anthraquinones	Absent	Present
12	Anthocyanins	Present	Present
13	Reducing Sugar	Present	Present

Quantitative Phytochemicals

The result of the quantitative phytochemical analysis is showed in Table 2. The result of the quantitative analysis showed that *S. nodiflora* contained abundant amount of the following compounds as follows alkaloids was 5.5 ± 0.01 mg/g), flavonoids content was 2.47± 0.01, tannins content of *S. nodiflora* was 11.6 ± 0.03 mg/g, saponin content was 0.143 ± 0.03, carbohydrate content was 21.11 ± 0.02, and the reducing sugar content was 1.65 ± 0.01 while steroids and terpenoids were absent (Table 2). Also, the result of the amount of phytochemicals present in honey showed that honey had 0.22 ± 0.01 amount of alkaloids, 17. 01 ± 0.02 of flavonoids, 0.67 ± 0.01 of tannins, 1.76 ± 0.01 saponins, 0.08 ± 0.002 steroids, 21.41 ± 0.07 terpenoids, 88.73 ± 0.33 carbohydrates and 12.12 ± 0.01 reducing sugar.

Table 2: Quantitative phyto-constituents in *S. nodiflora* and Honey

SN	Bioactive compound	*S. nodiflora*	Honey
1	Alkaloids	5.457 ± 0.01^{b}	0.244 ± 0.01^{a}
2	Flavonoids	2.747 ± 0.01^{a}	17.01 ± 0.02^{b}
3	Tannins	11.573 ± 0.03^{b}	0.669 ± 0.01^{a}
4	Saponins	0.143 ± 0.00^{a}	1.761 ± 0.01^{d}
5	Steroid	-	0.081 ± 0.002^{c}
6	Terpenoids	-	21.410 ± 0.066^{b}
7	Carbohydrates	21.113 ± 0.029^{a}	88.733 ± 0.333^{d}
8	Reducing sugar	1.65 ± 0.017^{a}	12.120 ± 0.001^{d}

(NB: Means having a common alphabet are not statistically different at the 5% level of significance).

Extractive Value *S. nodiflora*

The result of the extractive value of *S. nodiflora* using water, ethanol, methanol and hexane was presented in Table 3.0. The result further reveled that methanol (15.3%) gave greater yield of crude extract for *S. nodiflora* followed by aqueous (13.6%) and ethanol (2.9%) while hexane (2.60) had the lowest yield.

Table 3: Extractive Values of *S. nodiflora*

SN	Solvent	S. nodiflora
1	Water %	13.633 ± 0.06^{c}
2	Methanol %	15.267 ± 0.06^{d}
3	Ethanol %	$2.900 \pm 0.10a^{b}$
4	Hexane %	2.600 ± 0.10^{a}

(NB: Means having a common alphabet are not statistically different at the 5% level of significance).

Leaf Microscopy

The result of the leaf microscopy of *S. nodiflora* is presented in Plate 1.0. The result showed that both the adaxial and abaxial surface have irregular epidermal cell walls. Synedrella nodiflora leaf are irregular in shape with undulated anticlinal cell walls on both the adaxial (upper) and abaxial (lower) leaf surfaces. The leaf is hypostomatic (stomata only occur on the lower surface of the leaf. The stomata are anomocytic which implied absence of subsidiary cells and the guard cells are directly surrounded by epidermal cells. Also, there is presence of unicellular, unglandular trichome with conical base. The result of the transverse section of leaf (Plate 2) showed the presence of pith, xylem, phloem, palisade mesophyll, spongy mesophyll, pith.

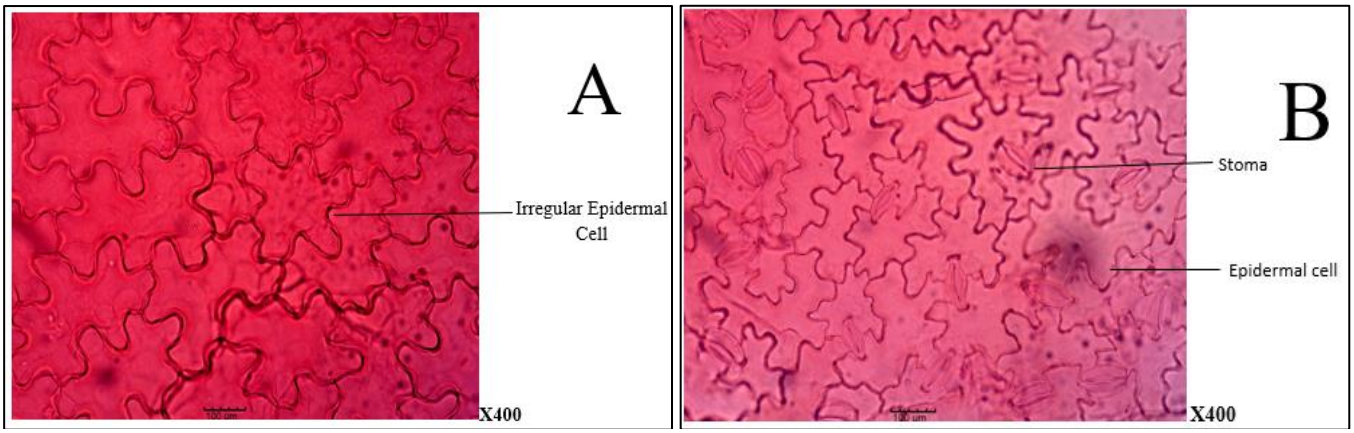

Plate 1: Leaf microscopy of *S. nodiflora* (A = Adaxial surface of the leaf of *S. nodiflora*; B = abaxial surface)

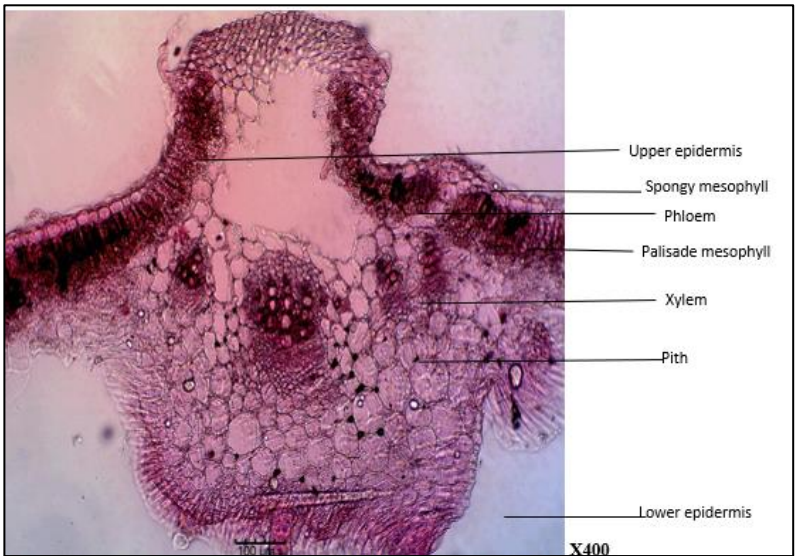

Plate 2: Transverse section of the leaf of *S. nodiflora*

Chemomicroscopy *S. nodiflora*

The result of the Chemomicroscopy is presented in Plate 2. Here, the Chemomicroscopy of *S. nodiflora* showed the presence of linin, starch, cystoliths, tannins and oil body while crystal of calcium oxalate was absent in the leaf powder sample.

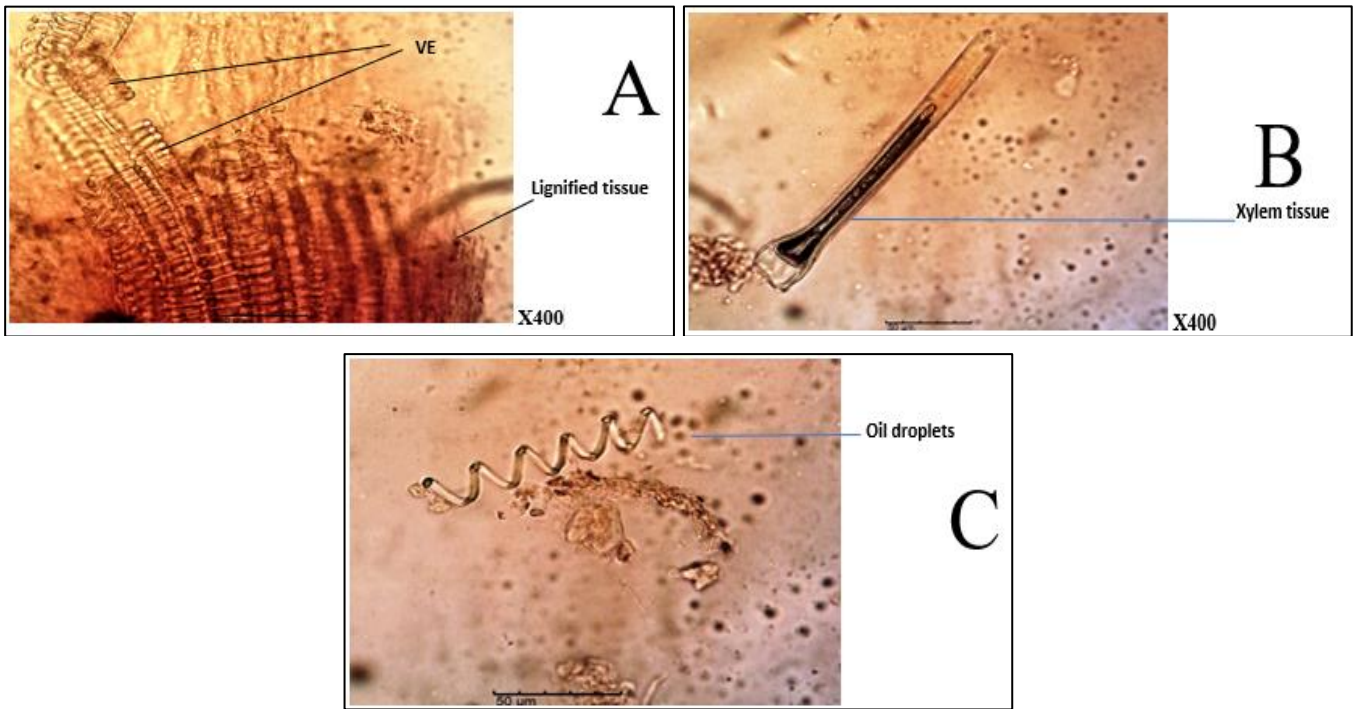

Plate 3: Chemomicrograph of the leaf powder of *S. nodiflora* ()

Proximate Profile

The result of proximate profile of honey is presented in Table 4.0. The result showed significant difference in the proximate content of *S. nodiflora* and honey. The result showed that *S. nodiflora* had 30.1% protein, 55% carbohydrates, 6.713% ash, 5.0% fibre and fats, respectively; and 4.7% moisture content while honey had 4.0% protein, 88% carbohydrates, 6% moisture, 1% ash and 1% fats, respectively.

Table 4: Proximate Profile of *S. nodiflora* and Honey

SN	Parameter	*S. nodiflora*	Honey
1	Protein %	30.063 ±0.37^{b}	4.060 ± 0.32^{a}
2	Ash %	6.713 ± 0.01^{b}	1.000 ± 0.00^{a}
3	Fiber content %	5.003 ± 0.01^{a}	-
4	Fat %	5.003 ± 0.01^{b}	1.000 ± 0.00^{a}
5	Moisture %	4.693 ± 0.01^{a}	6.207 ± 0.01^{b}
6	Carbohydrate %	55.237 ± 0.36^{a}	88.733 ± 0.33^{b}

(NB: Means having a common alphabet are not statistically different at the 5% level of significance).

Vitamin Profile of *S. nodiflora* and Honey

The vitamin profile of honey and S. *nodiflora* is presented in Table 5.0. The result showed significant difference in the vitamin profile of honey and *S. nodiflora*. It showed that S. *nodiflora* contained 0.8 mg/g retinol, 0.4 mg/g riboflavin and niacin, 15.7 mg/g pyridoxine, 6 mg/g biotin, 0.5 mg/g folate, 0.7 mg/g ascorbic acid and 0.03 mg/g tocopherol while honey contained 0.04 mg/g retinol, 0.07 mg/g riboflavin and Niacin, 12.0 mg/g pyridoxine, 0.6 mg/g biotin, 0.17 mg/g ascorbic acid and 0.033 mg/g tocopherol.

Table 5: Vitamin Profile of S. *nodiflora* and Honey

SN	Vitamin	*S. nodiflora*	Honey
1	Vit A / Retinol	0.810 ± 0.01^{b}	0.040 ± 0.01^{a}
2	Vit B2 /Riboflavin	0.403 ± 0.01^{b}	0.06 7 ± 0.01^{a}
3	Vit B3 /Niacin	0.357 ± 0.05^{b}	0.067 ± 0.01^{a}
4	Vit B6/Pyridoxine	15.710±0.31^{b}	12.060 ± 0.14^{a}
5	Vit B7 /Biotin	6.347± 0.02^{b}	0.603 ± 0.02^{a}
6	Vit B9/Folate	0.533 ± 0.01^{b}	0.170 ± 0.00^{a}
7	Vit C/Ascorbic acid	0.717 ± 0.03^{b}	0.170 ± 0.00^{a}
8	Vit K /Tocopherol	0.033 ± 0.01 ^{a}	0.033 ± 0.01^{a}

(NB: Means having a common alphabet are not statistically different at the 5% level of significance).

Mineral Profile of *S. nodiflora* and Honey

The result of mineral profile of *S. nodiflora* and honey is presented in Table 6.0. There was significant difference in the amount of minerals present in *S. nodiflora* and honey. It showed that S. *nodiflora* contained 2.5 mg/g magnesium, 37.7 mg/g calcium, 106.56 mg/g chlorine, 0.2 mg/g sodium and sulphate, 0.3 mg/g iron, 63.2 mg/g phosphorus, 0.4 mg/g manganese, 1 mg/g zinc, 0.2 mg/g potassium and cadmium respectively while honey had no content magnesium, 20.0 mg/g calcium, 88.75 mg/g chlorine, 0.1 mg/g sodium, 0.01 mg/g sulphate, 4.5 mg/g phosphorus, 0.3 mg/g manganese, 0.6 mg/g zinc, 0.02 mg/g potassium and 0.2 mg/g cadmium. This implied that *S. nodiflora* had greater content of mineral elements and their combination could serve as a good nutraceutical condiment.

Table 6: Mineral Profile of S. *nodiflora* and Honey

SN	Mineral element	*S. nodiflora*	Honey
1	Magnesium	2.483 ± 0.01^{a}	_
2	Calcium	37.730 ± 0.25^{b}	20.020 +0.02^{a}
3	Chlorine	106.570 ±0.06^{b}	88.750 ± 0.01^{a}
4	Sodium	0.203 ± 0.01^{b}	0.115 ± 0.08^{a}
	Sulphate	0.167 ± 0.01^{b}	0.013 ± 0.01^{a}
5	Iron	0.327 ± 0.01^{b}	0.023 ± 0.01^{a}
6	Phosphorous	63.230 + 0.14^{b}	4.520 ± 0.16^{a}
7	Manganese	0.347 ± 0.01 ^{b}	0.267 ± 0.01^{b}
8	Zinc	1.033 ± 0.02^{a}	0.633 ± 0.01^{b}
9	Potassium	0.183 ± 0.01^{b}	0.020 ± 0.00^{a}
10	Cadmium	0.190 ± 0.00^{b}	0.200 ± 0.00^{a}

(NB: Means having a common alphabet are not statistically different at the 5% level of significance while means having the same alphabet are statistically significant).

The Ash Value of *S. nodiflora*

The result of the ash value is presented in Table 3. The value of total ash was 15.0% while water soluble ash and acid insoluble ash were 10.07% and 5.0% respectively. The water loss on drying was 0.99% this and the value of ash content implies that the crude plant contained little impurities and contamination.

Antioxidant

The result of the anti-oxidant activity of crude methanol extract of *S. nodiflora* is presented in Table 7. The result showed significant difference (p<0.05) in the antioxidant potential of *S. nodiflora* crude extract and honey when compared to the standard (ascorbic acid) at 500, 250 and 125 µg/ml respectively. The result further showed that at 62.5 and 31.25 µg/ml the crude extract and honey had greater antioxidant activity than standard antioxidant drug and they were significantly different. The result showed that the standard drug had greater percentage reduction of oxidation at 500 (83.09%), 250 (79.66) and 125 (25%) µg/ml when compared to the crude methanol extract of *S. nodiflora* and honey and they were statistically different (p<0.05). Also, at 62.5 and 31.125 µg/ml, the crude extract had 18.91% and 16.19% inhibition of oxidation, respectively; while honey had 27.15% and 5.8%, respectively; when compared to the standard drug that had 11.75% and 4.37% respectively.

The result of the concentration of the crude extract and the fractions needed to decrease the initial DPPH concentration by 50% (IC_{50}) is also presented in Table 7.0. The result showed that the Ascorbic acid had the best IC_{50} value of 256.27 µg/ml followed by crude methanol extract of *S. nodiflora* with IC_{50} value of 520.29 µg/ml while honey had the least with IC_{50} value of 844.59 µg/ml.

Table 7: antioxidant profile of *S. nodiflora* and Honey

Dosage (µg/ml)	Standard /Ascorbic Acid	*S. nodiflora*	Honey
31.25	4.37^{a}	16.19^{b}	5.8^{a}
62.5	11.75^{a}	18.91^{b}	27.15^{c}
125	25.07^{bc}	22.49^{b}	16.12^{a}
250	79.66^{c}	33.38^{b}	11.6^{a}
500	82.09^{c}	38.32^{b}	25.86^{a}
IC50 (µg/ml)	256.28	520.29	844. 59

(NB: Means having a common alphabet are not statistically different at the 5% level of significance).

Cytoprotective potentials of *S. nodiflora* and Honey combination

Ulcer Index

The result of the cytoprotective profile of crude extract of *S. nodiflora* plus 1 ml honey combination is presented in Table 8.0. Here, there was significant reduction in ulceration of crude extract of *S. nodiflora* alone and the honey combination when compared to the negative control that received water alone. The result showed that the negative control group had ulcer index of 12.7 while the group treated with omeprazole had ulcer index of 6.63 and they are highly significantly different. Also, the group treated with crude extract at 100, 250 and 500 mg/kg of the crude extract alone had 7.0, 10.8 and 7.12 ulcer index respectively and they are significantly different when compared to the negative control group. Whereas, the group treated with the combination of 100, 250 and 500 mg/kg of the crude extract combined with honey had 3.12, 3.40 and 7.02 ulcer index respectively while honey alone had 3.5 ulcer index. This implied that the crude extract and the honey combination has improved anti-ulcer activity.

Percentage Inhibition of Ulceration

The result of the percentage inhibition of ulceration is presented in Table 8.0. The result showed that omeprazole group had 46% inhibition of ulceration while the group treated 100, 250 and 500 mg/kg of the crude extract had 43%, 12% and 42% inhibition of ulceration respectively. The group treated with I ml of honey combined with 100, 250 and 500 mg/kg of the crude extract showed enhanced reduction in ulceration though not significantly different when compared to the group treated with honey alone. The result further showed that 100, 250 and 500 mg/kg of the crude extract plus honey had 74.65, 72.32 and 42.78 inhibition of ulceration respectively.

Table 8: Cytoprotective profile of *S. nodiflora* and Honey combination

Groups /Treatment Mg/Kg	Ulcer Index	Percentage inhibition of Ulceration
Negative control Water 5ml/kg	12.27 ± 0.27^{e}	-
Omeprazole 50 mg/kg	6.63 ± 0.32^{c}	45.96^{b}
Normal Control	0.00 ± 0.00^{a}	100^{e}
SN 100mg/kg	7.00 ± 0.29^{c}	42.93^{b}
SN 250mg/kg	10.82 ± 0.40^{d}	11.84^{a}
SN 500mg/kg	7.12 ± 0.36^{c}	41.97^{b}
SN 100mg/kg + I ml Honey	3.12 ± 0.07^{b}	74.65^{d}
SN 250mg/kg + I ml Honey	3.40 ± 0.07^{b}	72.32^{c}
SN 500mg/kg + I ml Honey	7.02 ± 0.19^{c}	42.78^{b}
Honey 1ml per rat	3.46 ± 0.03^{b}	71.76^{c}

(NB: Means having a common alphabet are not statistically different at the 5% level of significance).

Antisecretory Effect of *S. nodiflora*

The result of the antisecretory assay is presented in Table 8, for effect of the extract combined with honey on gastric volume, gastric pH, free acidity and total acidity.

Effect of *S. nodiflora* on Gastric Volume

The effect of leaf extract of *S. nodiflora* on gastric volume is shown in Table 8. The result showed that the extract had a significant statistical effect ($P<0.005$) on the gastric volume at single doses of 100 (1.31ml), 250 (2.20 ml) and 500 mg/kg (2.80 ml) of the extract and their combination with honey when compared to the negative control (5.0). The result showed that the rats pretreated with crude leaf extract of *S. nodiflora* at 100, 250 and 500 mg/kg had gastric volume of 1.3, 2.2 and 2.8 respectively. It also showed that the rats pretreated with crude leaf extract of *S. nodiflora* at 100, 250 and 500 mg/kg combined with 1 mil of honey had gastric volume of 4.4, 3.7 and 2.9 respectively. This slight increase in the volume of gastric juice was attributed to increase in the volume of drug administered due to combination of the honey and crude extract.

Effect of *S. nodiflora* on Gastric pH

The result of the effect of leaf extract of *S. nodiflora* on gastric pH is presented in Table 9. The result showed that the extract at single doses of 100, 250 and 500 mg/kg of the extract and their combination with honey significantly ($P<0.005$) reduced gastric pH when compared to the negative control (3.0). The result showed that rats pretreated with crude extract at doses of 100, 250, and 500 mg/kg had pH of 3.3, 4.0, and 4.1 respectively, but when same doses in combination with honey, there was a significant reduction in the pH of gastric juice at doses of 100, 250, and 500 mg/kg with pH of 5.67, 5.67 and 6.0 respectively and this implied a synergistic effect on the pH values of the gastric when the crude extract is combined with honey.

Effect of *S. nodiflora* on Free acidity

The result of the effect of *S. nodiflora* on gastric free acidity is presented Table 9. The result showed that the extract at single doses of 100, 250 and 500 mg/kg of the extract and their combination with honey were statistically significant ($P<0.005$) in reduction of gastric free acidity when compared to the negative control (83). The result showed that the rats pretreated with crude leaf extract of *S. nodiflora* at 100, 250 and 500 mg/kg had gastric free acidity of 33.3, 51.67 and 43.3 respectively. However, when the crude extract at doses 100, 250, and 500 mg/kg were administered in combination with honey, there was a significant ($P<0.005$) reduction in the free acidity to 13.3, 6.67 and 30.0. This implied synergistic effect of the combination of honey and crude extract of *S. nodiflora* in reduction of free acidity of the gastric content.

Effect of *S. nodiflora* on Total Acidity

The effect of *S. nodiflora* on gastric total acidity is presented in Table 9. The result showed that the extract at single doses of 100, 250 and 500 mg/kg of the extract and their combination with honey were significantly different ($P<0.005$) in gastric total acidity when compared to the negative control (123). The result showed that the rats pretreated with crude leaf extract of *S. nodiflora* at 100, 250 and 500 mg/kg had gastric total acidity of 78.3, 110.0 and 113 respectively. However, when the crude extract at doses 100, 250, and 500 mg/kg were administered in combination with honey, there was a significant reduction in the total acidity to 56.67, 40.0 and 73.3 respectively while honey alone had 60. This implied synergistic effect of the combination of honey and crude extract of *S. nodiflora.*

Table 9: Antisecretory Profile of *S. nodiflora* and Honey combination

Groups /Treatment Mg/Kg	Gastric volume	PH	Free acidity	Total acidity
Negative control Water 5ml/kg	5.00±0.06^{cd}	3.00±0.00^{a}	83.33±3.33^{h}	123.33 ± 3.33^{f}
Cim 100mg/kg	1.00±0.22^{a}	6.00±0.00^{c}	0.00±0.00^{a}	54.00 ± 18.33^{b}
Normal Control	-	-	-	-
SN 100mg/kg	1.31±0.022^{a}	3.33±0.33^{a}	33.33±6.67^{e}	78.33 ± 11.67^{d}
SN 250mg/kg	2.20 ± 0.75^{ab}	4.00±0.58^{ab}	51.67±29.49^{g}	110.00 ± 32.15^{e}
SN 500mg/kg	2.80 ± 0.42^{b}	4.10±0.58^{ab}	43.33±6.67^{e}	113.33 ±17.64^{e}
SN 100mg/kg + Honey	4.40 ± 0.00^{c}	5.67±0.33^{c}	13.33±3.33^{c}	56.67 ± 3.33^{b}
SN 250mg/kg + Honey	3.70 ± 0.32^{c}	5.67±0.33^{c}	6.67±6.67^{b}	40.00 ± 5.77^{a}
SN 500mg/kg + Honey	2.90 ± 0.00^{b}	6.00±0.00^{c}	30.00±0.00^{d}	73.33 ± 3.33^{cd}
Honey 1ml per rat	4.40 ± 0.20^{c}	4.50±0.50^{b}	20.00±0.00^{c}	60.00 ± 0.00^{c}

(NB: Means having a common alphabet are not statistically different at the 5% level of significance).

Histopathology evaluation of stomach of the rats pretreated with the methanol leaf extract of *S. nodiflora* in the ethanol-induced ulcer models

The result of the histopathology is presented in plate A to J and the result showed a clear reduction in ulceration for all the treatment doses; single doses and combination of the crude extract and honey. The findings showed that at single doses of 100, 250, and 500 mg/kg (plate D to F) had mild to moderate ulceration when compared to the negative control (plate B) that received only distilled water which showed severe ulceration and marked depletion of the stomach gastric mucus. Also, rats pretreated with omeprazole (Plate C) showed reduction in ulceration, compared to the negative control (Plate B). Also, the result showed reduced ulceration in the rats pretreated with doses of 100, 250, and 500 mg/kg combined with honey when compared to the negative control. The histopathology results supported the result of the ulcer index and percentage inhibition of ulceration and this implied that leaf extract of *S. nodiflora*, when given orally either in combination or as single dose of 500 mg/kg, possessed anti-ulcer activity. This result is in agreement with the macroscopic evaluation and overall inhibition of ulceration indicated by the ulcer index, reduced gastric pH, and reduced free and total acidity.

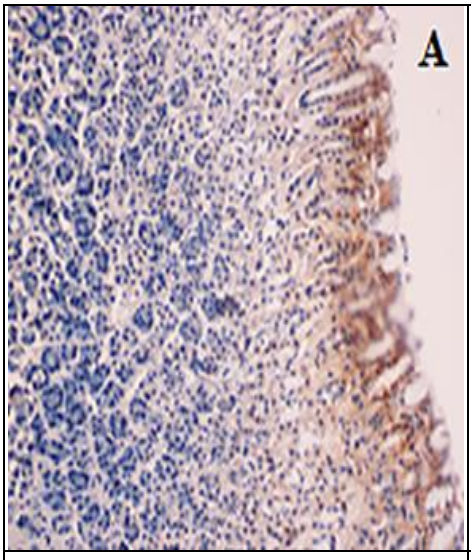

Plate A= the untreated control, showed typical gastric histoarchitecture with intact epithelium and glands

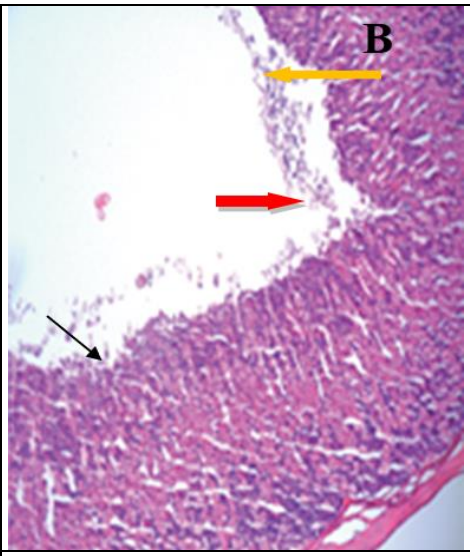

Plate B= negative control which showed a high degree of disruption of surface epithelial cells, necrotic lesions penetrate deeply into mucosa (red arrow), extensive edema of submucosal layer and leukocyte infiltration are present (yellow and black arrow). Inflammation as well as hemorrhagic patch formation are also on the gastric tissue. Histoarchitecture deeply affected

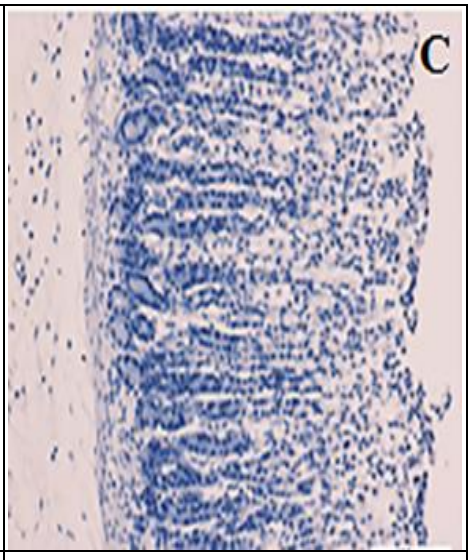

Plate C= positive control which showed slight disruption surface epithelial cells, no necrotic lesions, mucosa showed considerable reduction in inflammatory cells found in plate B

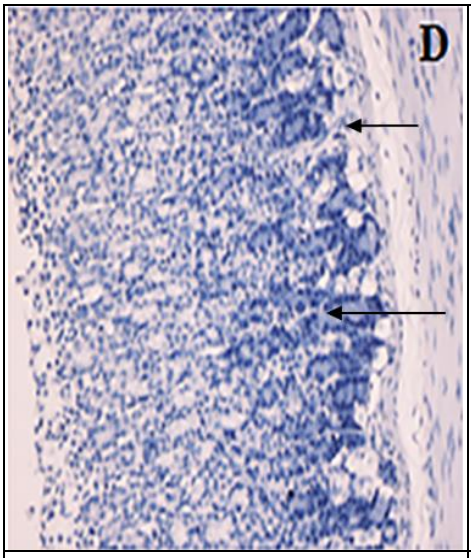

Plate D=100mg/kg of *S. nodiflora*, showed moderate disruption of the surface epithelium mucosa and mucosal damage (arrow) without submucosal and leukocytes infiltration (short arrow). Histoarchitecture slightly affected

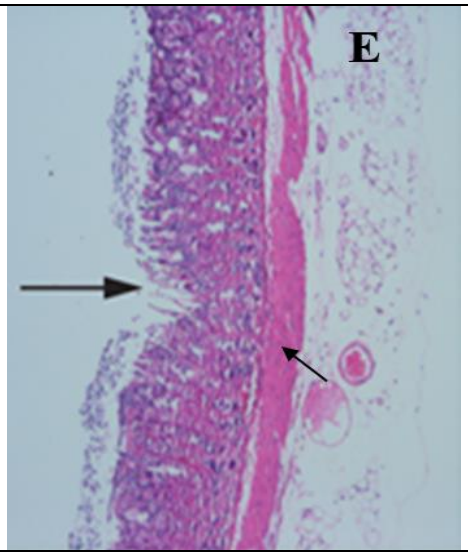

Plate E=250mg/kg of *S. nodiflora* showed mild appearance of hemorrhage, inflammation and moderate mucosal erosions by black arrows.

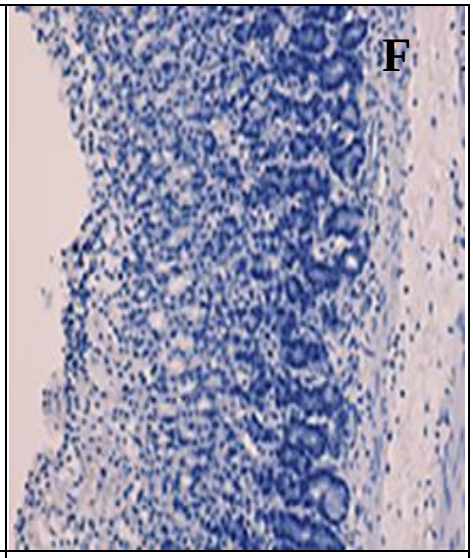

Plate F= 500 of *S. nodiflora*, showed mild disruption of the surface epithelium mucosa and slight mucosal damage (arrow) but no submucosal edema and infiltration are seen. Histoarchitecture slightly affected

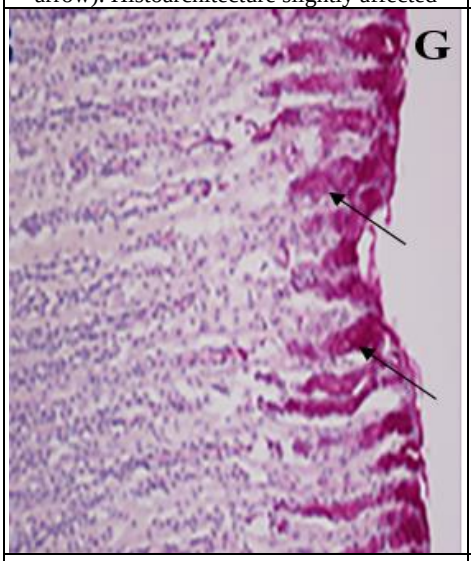

Plate G= 100mg *S. nodiflora* + 1 ml Honey, revealed attenuated morphological modifications, diminished inflammatory cell invasion (arrows) and mucosal preservation. Histoarchitecture slightly affected

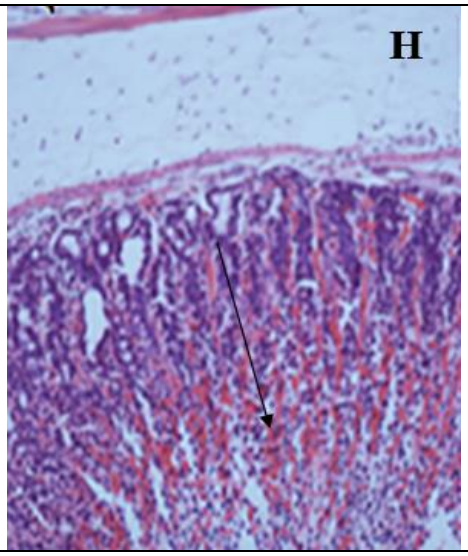

Plate H= 250mg *S. nodiflora* + 1 ml Honey, revealed mild disruption of the surface epithelium mucosa and slight mucosal damaged (arrow) but no submucosal edema and leukocytes infiltration are not seen.

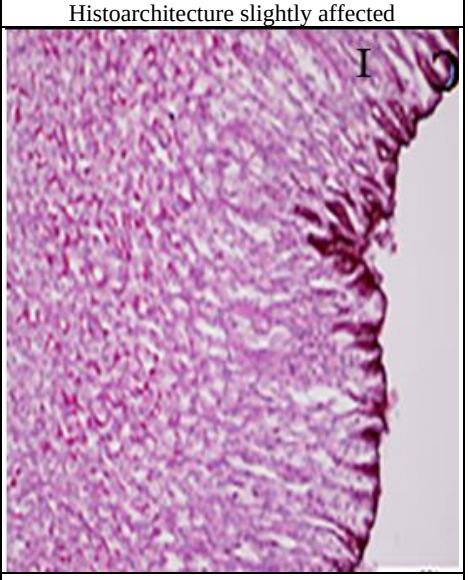

Plate I= 500mg *S. nodiflora* + 1 ml Honey, revealed moderate morphological modifications, diminished inflammatory cell invasion (arrows) and mucosal preservation. Histoarchitecture not affected

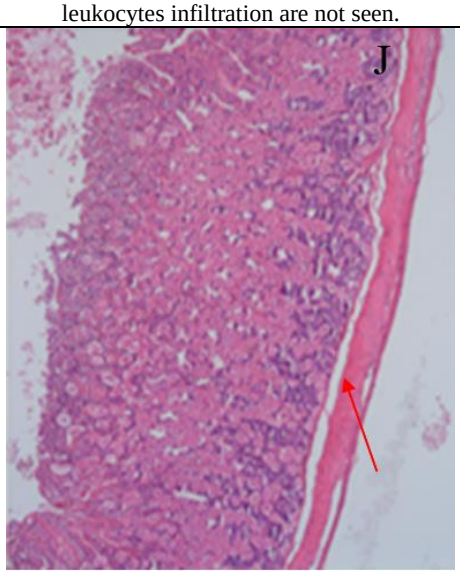

Plate J= honey alone, had a moderate distraction of the surface epithelium and necrotic lesion fully disappeared (arrow)

Discussion

The result of the fresh leaf microscopy showed unicellular, unglandular trichome with conical base. It also showed that both the adaxial and abaxial surface have irregular epidermal cell walls while anomocytic stomata is only present at the abaxial surface. This characteristic should be included in African Pharmacopeia for identification of *S. nodiflora*. The result of the phytochemical analysis of *S. nodiflora* and honey revealed a high concentration of tannins, flavonoids, and alkaloids, phytochemicals reported to have antioxidant and antiulcer activities and are collectively known as phenolics. Phenolics compounds have been reported to be well established and known for wound healing and anti-ulcer property. Previous studies have reported phenolics such as flavonoids, tannins and terpenoids to therapeutically possess antiulcer property [33, 34]. The mechanisms of the antiulcer activity were shown to be by protection of layer of the gastric mucosa by various mechanism of action and these includes cyto-protection, enhanced mucus re-epithelization, neovascularization, prostaglandin secretion, reduction of anti-angiogenic factors and enhanced antioxidant activity [34], and inhibition of proton pumps, thereby rendering the stomach less permeable and more resistant to chemical and mechanical injury caused by ulcerogens. This therapeutic action is similar to previous work by [35] where they reported antiulcer effect of crude methanol extract of *E. hirta* combined with honey in rats, these therefore implied that crude extract combined with honey showed great potency for cytoprotective and antioxidant activity which should be studied for commercial appliaction. Flavonoids on the other hand are known for antioxidant properties in addition to strengthening the mucosal defense system through stimulation of gastric mucus secretion and protection of the stomach lining through a cascade of endogenous and exogenous mechanisms.

The result of the findings showed that *S. nodiflora* contained abundance of essential elements such P, S, Cl, K and Ca and trace elements like Mn, Fe, Cu and Zn and these elements which are vital directly related to the maintenance of health and the proper functioning of the body. Manganese and Iron play vital roles in enzymes metabolism and immunomodulatory functions [36]. They play roles in secretion and synthesis of insulin in the islets of the pancreatic glands. Calcium is reported to play a role in the prevention and control of diseases [36], and may play vital role in the formation of bicarbonate which protects the mucus of the stomach through cell proliferation and regeneration of damaged gastric mucosa caused by the ulcerogenic factor such as ethanol. Copper functions as a precursor of many enzymes and proteins, and are vital in the growth, blood cell production and the transport and metabolism of Fe while also interacting other elements, especially Zn, leading a competition for transporters [36].

Sulphate plays a vital role as a precursor and essential components of many amino acids, enzymes and other biomolecules some of which play vital roles as antioxidants and this makes *S. nodiflora* an important source of these elements [38]. Therefore, we recommend that micro sulphate should be added in the standardization of phytomedicine. Sulphate participates in almost all metabolic processes as part of structural molecules and plays vital roles as component in enzymatic reactions [39], responsible for the growth and renewal of tissues [37]. The zinc is present in all of the tissues of the human body including the mucosa tissue and the anion balance plays vital role in the protection of the body and therefore Zn is an important factor in the healing of gastric lesions, especially in the initial phases [40]. Zinc plays important role as a component of various enzymes and other macro-molecules which plays significant role in maintenance of health [36].

The antioxidant and antiulcer activities could be attributed to the presence of alkaloids, tannins, flavonoids and saponins and enhanced role of manganese, magnesium, zinc, calcium and potassium in metabolism, secretion and utilization of bicarbonate ions and mucus of the parietal cells of the stomach and this therefore suggest manganese, magnesium, zinc, calcium and potassium in *S. nodiflora* played important in maintenance of anion and cation balance of the stomach and therefore should be included in the standardization of *S. nodiflora* and other medicinal plants for the treatment of antioxidants and ulcer.

Conflicts of interest

The authors declare that there is no conflict of interest among the authors.

Author contributions: All authors contributed to the conceptualization, investigation, methodology, supervision, formal analysis, and writing of the manuscript.

Reference

1. Surh YJ, Ferguson LR, Dietary, medicinal antimutagens and anticarcinogens: molecular mechanisms and chemopreventive potential--highlights of a symposium. Mutat Res 2003;523-524:1-8. doi: 10.1016/s0027-5107(02)00343-3. PMID: 12628498
2. Aruoma OI. Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. Mutat Res 2003;523-524:9-20. doi: 10.1016/s0027-5107(02)00317-2. PMID: 12628499.
3. Lü JM, Lin PH, Yao Q, Chen C. Chemical and molecular mechanisms of antioxidants: experimental approaches and model systems. J Cell Mol Med. 2010;14(4):840-60. doi: 10.1111/j.1582-4934.2009.00897. x. Epub 2009. PMID: 19754673; PMCID: PMC2927345.
4. Vajragupta O, Boonchoong P, Berliner LJ. Manganese complexes of curcumin analogues: evaluation of hydroxyl radical scavenging ability, superoxide dismutase activity and stability towards hydrolysis. Free Radic Res 2004;38(3):303-14. doi: 10.1080/10715760310001643339. PMID: 15129738.
5. Adjibode AG, Tougan UP, Youssao AKI, Mensah GA, Hanzen CH, Koutinhouin GB. *Synedrella nodiflora* (L.) Gaertn: a review on its phytochemical screening and uses in animal husbandry and medicine. International Journal of Advanced Scientific and Technical Research 2015;5(3),
6. Li Z, Qiao Y, Li J, An C, Hu K, Tang M. Acute and sub-chronic toxicity studies of the extract of Thunberg Fritillary Bulb. Regul Toxicology Pharmacol 2014;68:370-7;
7. Odom MD, Richard B, William DJ, Timothy GB. Andrews' diseases of the skin: clinical dermatology. W.B. Saunders Company 2000,1135;
8. Koutinhouin GB, Tougan UP, Kpodékon TM, Boko C, Goudjihounde SM, Aoulou A *et al*. Valuation of *Synedrella nodiflora* leaves as feed supplement for rabbit reared in a humid tropical environment: impact on reproductive performance. International Journal of

Agronomy and Agricultural Research (IJAAR), 2014;5(4):55-64;
9. Amoateng P, Woode E, Kombian SB. Anticonvulsant and related neuropharmacological effects of the whole plant extract of *Synedrella nodiflora* (L.) Gaertn (Asteraceae). J Pharm Bioall Sci 2012;4:140-8.
10. Woode E, Amoateng P, Abotsi WM. Ethopharmacological analysis of the effects of the whole plant extract of *Synedrella nodiflora* (L.) Gaertn (Asteraceae) in murine models. Der Pharmacia Sinica; 2011;2:54-67;
11. Amoateng P, Assumeng Koffuor G, Sarpong K, Oteng Agyapong K. Free radical scavenging and anti-lipid peroxidative effects of a hydro-ethanolic extract of the whole plant of *Synedrella nodiflora* (L.) Gaertn (Asteraceae). Free Rad Antiox 2011;1:70-8.
12. Zahan R, Nahar L, Haque A, Mosaddik A, Fazal A, Alam Z, *et al*. Antidiarrhoeal and hypoglycemic effects of *Synedrella nodiflora*. Phytopharmacology 2012;2(2):257-264;
13. Woode E, Amoateng P, Ansah C, Duwiejua M. Anti-nociceptive effects of an ethanolic extract of the whole plant of *Synedrella nodiflora* (L.) gaertn in mice: Involvement of adenosinergic mechanisms. J Pharm Toxicol 2009;4:17-29.
14. Idu M, Onyibe HI. Medicinal plants of Edo state, Nigeria. Res J Med Plant 2007;1:32-41
15. Mshana NR, Abbiw DK, Addae-Mensah I, Adjanohoun E, Ahyi MR, Enow-Orock EG. Traditional medicine and pharmacopoeia. Contribution to the revision of ethnobotanical and floristic studies in Ghana. Scientific, Technical and Research Commission of the Organization of African Unity Accra: Institute for Scientific and Technological Information. 2000, 122.
16. Dalziel JM. The hairs lining the loculi of fruits of species of Parinarium. London: Proc Linn Soc.1931, 99.
17. Cobbinah JR, Moss C, Golob P, Belmain SR. Conducting ethnobotanical surveys; an example from Ghana on plants used for the protection of stored cereals and pulses. In: NRI Bulletin. 77. Chatham: Natural Resource Institute, 1999.
18. Sumi W, Ting KN, Kho TJ, Lim KH. Antibacterial and antioxidant activities of *Synedrella nodiflora* (L) Gaertn. (Asteraceae). Journal of Complementary and Integrative Medicine 2011;8(1):1-13.
19. Trease GE, Evans WC. Pharmacognosy, 11th edition. Bailliere Tindall, London 1989, 45-50.
20. Harborne JB. Phytochemical Methods 3rd ed. Chapman and Hall Ltd 1973, 135-203.
21. Singleton VL, Rossi JA. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. American journal of Enology and Viticulture 1965;16:144-158.
22. Marinova D, Ribarova F, Atanassova M. Total Phenolics And Total Flavonoids in Bulgarian Fruits and Vegetables. Journal of the University of Chemical Technology and Metallurgy 2005;40(3):255-260.
23. Sofowora A. Medicinal Plants and Traditional Medicine in Africa'. 3rd edn. Spectrum Books, Ibadan 2008.
24. Ghorai N, Chakraborty S, Gucchait S, Saha KS, Biswas B. Estimation of total Terpenoids concentration in plant tissues using a monoterpene, Linalool as standard reagent. Nature Protocol Exchange 2012. https://doi.org/10.1038/protex.2012.055.
25. Nwafor FI, Nwosu MO, Nwafor AZ. Taxonomic and Ecological Significance of Foliar Epidermal Characters in Four Taxa of *Mussaenda* L. (Rubiaceae) in Nigeria. Annual Research & Review in Biology, 2019;32(5):1-12.
26. Lorke D. A new approach to practical acute toxicity testing. Archives of Toxicology 1983;54(4):279-287.
27. Zimmerman M. Ethical guidelines for investigation of experimental pain in conscious animals. Pain 1983;16:109-110.
28. AOAC. Official Methods of Analysis of the Analytical Chemists 18th Edition, Washington, D. C. U. S. A. 2010.
29. Garg GP, Nigam SK, Ogle CW. The gastric antiulcer effects of the leaves of the neem tree. Planta Med. 1993; 59:215-217. https://doi.org/10.1055/s-2006-959654.
30. Umeokoli BO, Ekeh MN, Eze PM, Umeyor CE, Abba CC. Improved gastric lesion of ulceronic Mice treated with bark extract and fractions of Newbouldia laevis. African Journal of Pharmacy and Pharmacology 2015;9(21):553-560. https://doi.org/10.5897/AJPP2015.%204331.
31. Shay H, Komarov SA, Fels SE, Meraze D, Gruenstein M, Siplet H. A simple method for the uniform production of gastric ulceration in rat. Gastroenterol 1945;5:43-61.
32. Larson H, Carlson E, Junggren U, Olbe L, Sjostrand SE, Skanberg I *et al*. Inhibition of Gastric acid Secretion by Omeprazole in the Dog and Rats. Gastroentology 1983;85:900-907.
33. Zhong SM, Waterman PG, Jeffreys JAD. Naphtoquinones and triterpenes from African *Diospyros* species. Phytochemistry 1984;23:1067-1072.
34. Recio MC, Giner RM, Máñez S, Gueho J, Julien HR, Hostettmann K *et al*. Investigations on the steroidal anti-inflammatory activity of triterpenoids from *Diospyros leucomelas*. Planta Med. 1995;61(1):9-12. doi: 10.1055/s-2006-957988. PMID: 7701004.
35. Onyeka IP, Bako SP, Suleiman MM, Onyegbule AF, Morikwe UC, Ogbue CO. "Antiulcer Effects of Methanol Extract of *Euphorbia hirta* and Honey Combination in Rats", BioMed Research International 2020, Article ID 6827504, 8 pages, 2020. https://doi.org/10.1155/2020/6827504.
36. Marmiroli, Maestri Marmiroli N, Maestri E. Health implications of trace elements in the environment and food chain. In: Prasad MNV, editor. Trace elements as contaminants and nutrients: consequences in ecosystems and human health. Wiley; Hoboken 2008, 23-53.
37. Desideri, Meli, Roselli. Desideri D, Meli MA, Roselli C. Determination of essential and non-essential elements in some medicinal plants by polarized X ray fluorescence spectrometer (EDPXRF) Microchemical Journal 2010;95(2):174-180. doi: 10.1016/j.microc.2009.11.010.
38. Komarnisky LA, Christopherson RJ, Basu TK. Sulfur: its clinical and toxicologic aspects. Nutrition 2003;19(1):54-61. doi: 10.1016/s0899-9007(02)00833-x. PMID: 12507640.
39. Nielsen. Nielsen FH. Major minerals—calcium, magnesium and phosphorus. In: Driskell JA, editor. Nutrition and exercise concerns of middle age. Taylor & Francis; Boca Raton: 2009, 193-218.
40. Watanabe T, Arakawa T, Fukuda T, Higuchi K, Kobayashi K. Zinc deficiency delays gastric ulcer healing in rats. Digestive Diseases and Sciences 1995;40(6):1340_1344. DOI 10.1007/BF02065548.