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Original Research Article

Evaluation of the antiulcer and antimicrobial activities of methanol leaf extract of *Helianthus annuus*

Innocent F. Ajegi[1], Gloria O. Ajegi[1], Obinna C. Ajaegbu[1], Matthew O. Nwokike[2], Mansur A. Ramalan[3], Victor I. Eje[4], Godwin C. Akuodor[1]*

[1]Department of Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Nigeria
[2]Department of Pharmacology, Prince Abubakar Audu University, Anyigba, Nigeria
[3]Department of Pharmacology and Therapeutics, Bayero University, Kano, Nigeria
[4]Department of Physiology, Federal University of Health Sciences, Otukpo, Nigeria

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***Correspondence:**
Dr. Godwin C. Akuodor,
Email: goddyakuodor@yahoo.com

ABSTRACT

Background: Medicinal plants are widely used in treating and preventing specific diseases and are known to play an important role in health care. *Helianthus annuus* is one of such plants whose therapeutic applications no doubt have numerous folkloric background. This study aimed at assessing *Helianthus annuus* methanol leaf extract for antiulcer and antimicrobial potentials
Methods: The antiulcer activity was evaluated using aspirin, ethanol and histamine methods in Wistar rats, while the antimicrobial activity was carried out on selected microorganisms. The acute toxicity test and phytochemical screening of the extract were also conducted.
Results: The methanol leaf extract exhibited significant ($p<0.05$ and $p<0.01$) antiulcer effect in both model. The ulcer protection effect of the extract is comparable to omeprazole. The extract also significantly inhibited the growth of test organisms. The acute toxicity test produced no lethality in rats, whereas the phytochemical analysis revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids and steroids.
Conclusions: The findings confirm the ethnomedicinal use of *H. annuus* leaf as a valuable natural agent for the treatment of ulcer and microbial infections.

Keywords: *Helianthus annuus*, Leaf, Aspirin, Histamine, Microbes

INTRODUCTION

Medicinal plants are used commonly by most people in rural areas in treatment of diseases traditionally and they are considered beneficial in healthcare. Medicinal plants are generally, considered to possess innumerable bioactive constituents which steadily increase their demand as an alternative to synthetic agents, leading to an increase in their demand as natural products.[1-4] More so, formulated products derived from these plants are safer with less harmful effect for human than the synthetic drugs.[5] However, pharmacological evaluation of these agents is important for drug development. Hence, plants with medicinal values remain the major basis upon which new chemical compounds are discovered and this eventually results into new drugs.

Helianthus annuus has been noted for its medicinal and nutritional usage worldwide. *H. annuus* (Asteraceae) commonly referred to as sunflower is cultivated in America, Africa, Asia and Australia. It is commonly called

sunflower is an annual perennial plant belonging to the family of Astereacea which is widely distributed.[6] Its folklore medicinal uses in many ways have been documented.[7] It is a perennial plant with many pharmacological properties. The anti-inflammatory, analgesic, antihyperglycaemic, antioxidant, hepatoprotective, antifertility, and antimicrobial activities of H. annuus have been documented.[8-10] *H. annuus* leaves, decoctions and infusions are used in folkloric medicine as diuretic, antidiabetic, expectorant, gastrointestinal stimulant, antimicrobial and analgesic agents.[11,12] The leaves of *H. annuus* are endowed with pharmacological activities such as antispermatogenic, analgesic and anti-inflammatory, antidiabetic, antioxidant, antifertility, antidiarrheal, antihistaminic, antilithiatic and antimicrobial activities.[13,14] The present study, therefore, evaluated the antiulcer and antimicrobial potential of *Helinthus annuus* methanol leaf extract to justify its traditional use in ulceration and microbial conditions.

METHODS

Plant material collection and authentication

Fresh leaves of *Helianthus annuus* were collected from the botanical garden of the department of agricultural economics, federal polytechnic Nekede and authenticated by Dr. Clinton Emekoma a botanist with above department and institution. The leaves were washed thoroughly, air-dried for about three weeks and pulverized to powder. The powder was divided into equal parts of 300 g each.

Preparation of plant and extraction procedure

Maceration method was used in the preparation of the extract. Each of 300 g of the powdered leaf was introduced into separate glass containers. The first portion was thoroughly extracted with 2.5 litres of methanol using cold maceration. It was allowed to stand for 48 hours with intermittent agitations, it was then filtered and thereafter the material was washed with methanol in a stepwise manner until the solution became faint green. The second portion was exhaustively extracted with n-hexane, ethyl acetate and then methanol in order of increasing polarity. Approximately 1.5 litres each of the solvents was added to each phase and then allowed to stand for 48 hours with intermittent agitations and subsequently filtered. The marc was removed and spread on a clean nylon mat for complete evaporation of previous solvent before re-introducing it into the container for subsequent extraction.

Experimental animals

Adult Wistar rats (200-250 g) were procured from the Animal House of the Department of Physiology, Faculty of Medicine and Surgery, Nnamdi Azikiwe University, Awka. They were allowed free access to water and livestock feeds throughout the experimental periods. The animals were acclimatized for about 14 days and maintained on light/dark cycle of 12 hours each. The animals were fed on standard feed and water *ad libitum*. The study protocol was carried out as per the rules of the National institute of health guide for the care and use of laboratory animals.[15]

Phytochemical analysis

The whole methanol extract and the various fractions were subjected to phytochemical analysis to identify the phytoconstituents of extracts using standard methods.[16,17]

Acute toxicity test

This was determined following the method described by Lorke, 1983.[18] The study was carried out in two phases. In the first phase, nine mice were divided into three groups of three mice each. They were given 10 mg/kg, 100 mg/kg and 1000 mg/kg of the leaf extract respectively. They were then monitored for signs of toxicity initially for first 4 hours, and then for 24 hours. The signs of toxicity that were looked out for include hyperactivity, paw licking, respiratory distress, and mortality. At the end of the first phase, there was no mortality. The study then proceeded to the second phase. In this phase, three mice were grouped into three with one mouse in each group, and given 1600 mg/kg, 2900 mg/kg and 5000 mg/kg of the extract respectively, and then monitored for signs of toxicity as stated earlier. The animals were further monitored for 48 and 72 hours for signs of late toxicity.

Antiulcer activity test; aspirin induced ulceration in rats

The rats employed for the study were deprived of food for 48 h but had access to water ad libitum. They were divided into five of six in each cage. They were randomly divided into 7 groups of 5 per group. Group 1 and 2 were administered distilled water (10 mL/kg) and omeprazole (20 mg/kg) respectively. Group 3, 4 and 5 were administered (100 mg/kg, 200 mg/kg and 400 mg/kg) of the methanol leaf extract of *Helianthus annuus*. Thirty min later ulcer was induced by oral administration of 150 mg/kg of Aspirin to all the groups. The animals were sacrificed 4 h after and their stomachs opened along the greater curvature and washed to remove gastric contents and examined under a dissecting microscope with square-grid eye piece to assess the formation of ulcers.[19] For each stomach, ulcerated and total areas were measured as mm2.

Ethanol induced ulceration in rats

The experimental rats were fasted for 24 h prior to the study. They were randomly divided into five groups of six per group. Group 1 and 2 were administered distilled water (10 ml/kg) and omeprazole (20 mg/kg) respectively. Group 3, 4 and 5 were administered (100 mg/kg, 200 mg/kg and 400 mg/kg) of the methanol leaf extract of *Helianthus annuus.* All administered orally. Thirty minute later, ulceration was induced by intragastric instillation of 1 mL of absolute (99 %) ethanol. One hour after, rats were sacrificed under ether anaesthesia and their stomachs

removed and opened along the greater curvature to macroscopically examine the lesions.[20] The number, length and severity of the erosions were noted and scored.[21]

Histamine induced ulcer in rats

The animals were fasted for 18 h prior to the experiment but had water *ad libitum*. They were randomly divided into five groups of six per group. Group 1 and 2 were administered normal saline (10 ml/kg) and ranitidine (20 mg/kg) respectively. Group 3, 4 and 5 were administered (100 mg/kg, 200 mg/kg and 200 mg/kg) of the methanol leaf extract of *H. anuus*. All administrations were carried out orally. Thirty minutes after pretreatment, ulceration was induced by subcutaneous administration of 100 mg/kg of histamine.[22] After 2 hours, the animals were sacrificed and stomach removed and opened along the greater curvature. The stomach was rinsed under a stream of water and pined flat on a corkboard. The stomach mucosa was examined with a hand lens of (×10) magnification. The number, length and severity of the erosions were noted and scored.[23]

Antimicrobial screening.

Five microbial agents were used in the study. These include *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Candida albicans* and *Aspergillus*. All the microorganisms were gotten from the microbiology department of the Nnamdi Azikiwe university teaching hospital. Nnewi and were maintained on nutrient broth agar and Saboraud's Dextrose agar at 4°c respectively. Before use they were sub-cultured in nutrient broth agar and Saboraud's Dextrose agar plates at 37°c for 24 hours. The agar disc diffusion method was employed.[24] Using a sterile pipette 0.1 ml 1.0×10^6 organism/ml suspension of microorganism was placed at the centre of petri dish and 50 ml of molten nutrient broth agar poured on it. This dish was agitated gently in a centrifugal direction to ensure even distribution of the seeded organism. This was then allowed to stand so as to solidify. Thereafter, two drops of 0.02 ml of the extracts were applied to appropriately labeled wells about 6 mm made in gelled agar containing 1.0×10^6 organism/ml. The plates were incubated at 37°C for 24 hours for bacteria and at 25°C for 72 hours for fungi. The effect of the extract of *H. annuus* on the growth of the microorganism were studied by observing the zones of inhibitions. The experiment was carried out in triplicates and the average clear diameter of zones of inhibition was recorded in each case.

Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of the extract was assayed through micro dilution method.[25] Ethanol root bark extract of *H. annuus* which inhibits growth of one or more microorganisms was examined for MIC. Serial dilutions of the extract were prepared to the concentrations of 6.25, 12.5, 25, 50, 100 and 125 µg/ ml. The wells for the test were inoculated with 0.1 ml aliquot containing the test organisms (1x106 cfu/ml) and serial dilution of the root bark extract containing 50 µl each. Thereafter, micro plate was incubated at 37°C for 24 hours and dilutions of the extract corresponding to each test organism showing no visible growth was considered as the MIC.

Statistical analysis

Data were expressed as mean± SEM and analyzed with statistical package for social sciences (SPSS version 20), using one-way analysis of variance (ANOVA), followed by Dunnett's test. A difference in the mean $p<0.05$ was considered as statistically significant.

RESULTS

Phytochemical analysis

Phytochemical evaluation of *H. annuus* leaf extract showed the presence of the following secondary metabolites; alkaloids, saponins, tannins, flavonoids, terpenoids, sterols and phenols.

Table 1: Effect of methanol leaf extract of *H. annuus* on aspirin induced ulcers in rats.

Groups	Dose	Ulcer index (UI)	% protection of ulceration
Distilled water	10 ml/kg	4.33±0.21	-
Omeprazole	20 mg/kg	0.00±00	100[b]
H. annuus	100 mg/kg	1.55±1.26	64[a]
	200 mg/kg	0.90±2.33	79[a]
	400 mg/kg	0.00±0.00	100[b]

Results are expressed as mean±SEM; (n=5), by one-way ANOVA followed by Dunnett's test (compared with control group) [a]$p<0.05$, [b]$p<0.01$.

Table 2: Effect of methanol leaf extract of *H. annuus* on ethanol induced ulcers in rats.

Groups	Dose	Ulcer index (UI)	% Protection of ulceration
Distilled water	10 ml/kg	3.45±0.21	-
Omeprazole	20 mg/kg	0.00±00	100[b]
H. annuus	100 mg/kg	0.45±2.17	87[b]
	200 mg/kg	0.00±00	100[b]
	400 mg/kg	0.00±0.00	100[b]

Results are expressed as mean±SEM; (n=5), by one-way ANOVA followed by Dunnett's test (compared with control group) [a]$p<0.05$, [b]$p<0.01$.

Acute toxicity test

There were no lethality or toxic reactions observed at any of the doses administered. All the mice were healthy and active during and after the period of study. Hence, oral acute toxicity result was greater than 5000 mg/kg in mice.

Effect of H. annuus methanol leaf extract on aspirin induced ulcers

The results obtained with the extract in this experimental model are shown in (Table 1). *H. annuus* provided marked protection at the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg with protection in ulcer index of 64%, 77% and 100% respectively. The standard drug, Omeprazol, showed a protection of 92.38% (Table 1).

Table 3: Effect of aqueous leaf extract of *H. annuus* on histamine induced ulcers in rats.

Groups	Dose	Ulcer index (UI)	% Protection of ulceration
Distilled water	10 ml/kg	3.52±2.30	-
Omeprazole	20 mg/kg	0.00±00	100[b]
H. annuus	100 mg/kg	0.36±3.11	90[b]
	200 mg/kg	0.00±00	100[b]
	400 mg/kg	0.00±0.00	100[b]

Results are expressed as mean±SEM; (n=5), by one-way ANOVA followed by Dunnett's test (compared with control group) [a]p<0.05, [b]p<0.01

Table 4: Zone of inhibition (mm) of organisms by methanol leaf extract of *Helianthus anuus* and gentamicin organisms.

Zone of inhibition (mm)		
Organisms	*H. anuus* extract	Gentamicin
S. aureus	20	24
K. pneumonia	18	20
C. albicans	*19*	10
Aspergillus niger	17	11
E. coli	20	17

Effect of H. annuus methanol leaf extract on ethanol induced gastric ulcers

Pre-treatment of rats with the methanol extract at doses employed (100 mg/kg, 200 mg/kg and 400 mg/kg) produced 87%, 100% and 100% protection against gastric mucosal damage, while the standard, omeprazole 20 mg/kg exhibited 100% protection under the same condition (Table 2).

Effect of H. annuus methanol leaf extract on histamine induced ulcers

In water immersion stress-induced ulcer model, *H. annuus* methanol leaf extract (100 mg/kg, 200 mg/kg and 400 mg/kg) showed protection index of 90%, 100% and 100% respectively, whereas Omeprazole, exhibited 100% protection (Table 3).

Antibacterial activity

The antimicrobial activity of *H. annuus* leaf extract is presented on (Table 4). The extract showed activity against all the organisms tested. Gentamycin tested at 10 µg/ml, inhibited three of the organisms, while *C. albicans* and *Aspergillus niger* were moderately inhibited.

Minimum inhibitory concentration

The MIC of ethanol root bark extract of *H. annuus* against the organisms were obtained as follows: *S. aureus* (20), *K. pneumonia* (22), *C. albicans* (35), *Aspergillus niger* (40) and *E. coli* (25).

DISCUSSION

Traditional folklore medicine has claimed a lot of success in the management of peptic ulcer disease and this has informed the recent screening of *Helianthus annuus* for antiulcer properties with the aim of isolating potent and safer antiulcer drugs from medicinal plants. Due to the reported side effects of available anti-ulcer drugs, focus has been shifted towards natural products as the new sources of anti-ulcer agents. With the growing interest in natural medicine, various plants have been studied based on the traditional knowledge of their pharmacological properties and confirmed to be useful in treating and managing ulcers.[26] In addition, medicinal plants have been known to be amongst the most attractive sources of new drugs, and have been shown to give promising results in treatment of various diseases including gastric and duodenal ulcers.[27]

Aspirin is a potent cyclooxygenase inhibitor which suppresses gastroduodenal bicarbonate secretion, reduces endogenous prostaglandin biosynthesis and disrupts the mucosal barrier as well as mucosal blood flow.[28] Aspirin increases acid secretion and produce microvasculature damage by generation of free radicals. It is well known that inhibition of prostaglandin synthesis which is essential for mucosal integrity and regeneration will trigger the mucosal lining damage. However, it is believed that *H. annuus* leaf extract exert its antiulcer activity by increasing the synthesis of endogenous prostaglandins, which in turn promote mucus secretion and enhance the mucosal barrier against the actions of various damaging agents. The pathogenesis of ethanol-induced gastric damage in rats involves superficial aggressive cellular necrosis as well as the release of tissue derived mediators such as histamine and leucotriene C4. These mediators act on gastric microvasculature, triggering a series of events that lead to mucosal and sub mucosal damage.[29] The pre-treatments with *H. annuus* significantly inhibited gastric lesions produced by acidified ethanol. The fact that the extract protected the gastric mucosa of rats against ethanol-

induced acute mucosal damage with a reduction of the ulcer index indicates that H. annuus leaf extract could be an effective gastroprotective agent. The mechanism for the mucosal protective action of this extract may be due partly to the stimulation of PG synthesis since endogenous PGs play a crucial role in gastroprotection.[30] It is also possible that an increase in gastric mucus or a possible leukotriene antagonism may contribute to the gastroprotective effect of this plant extract.[31] Histamine-induced gastric ulcers has long been recognized and mediated through stimulation of H2 receptors which results in enhanced gastric acid secretion and vasodilatation.[32] Histamine not only enhances gastric acid secretion, but also causes disturbances of the gastric mucosa, microcirculation, abnormal motility and reduction in mucus production. The methanol extract of *H. annuus* significantly protected the animals from ulceration. This may be due to inhibition of contractile response of the intestine and blockade of histamine H_2 receptors on the parietal cells leading reduced gastric acid and pepsin output. The gastroprotective effect exhibited by *H. annuus* may be attributed to the presence of flavonoids and polypheolic compounds, saponins and tannins.[33,34] These compounds most likely inhibit gastric mucosal injury by scavenging the stress-generated oxygen metabolites.[35] Flavonoids have been reported possess protective effects on gastric mucosa and a variety of ulcerogenic agents in different mammalian species.[36] Hence, many studies have examined the antiulcerogenic activities of plants containing flavonoids. Plants containing flavonoids were found to be effective in preventing this kind of lesion, mainly because of their antioxidant properties. Microbial colonization of the gastro-intestinal system has been associated with a variety of peptic ulcer diseases.[37] *Helicobacter pylori* have been implicated as the microorganism involved in the pathogenesis of peptic ulcer disease and has made the use of antibiotics imperative in peptic ulcer disease management. *Helicobacter pylori* eradication provides a definitive cure than the contemporary palliative symptom alleviation.[38,39] Although, *Helicobacter pylori* could not be cultured, the effects of the whole methanol extract against other gram-negative pathogens belonging to the same class as *Helicobacter pylori* were tested. The methanol leaf extract showed significant inhibition of microbial growth. The findings have shown that the extract could possess antagonizing qualities capable of preventing microbial survival.

CONCLUSION

In conclusion, the present study has shown that the methanol leaf extract of *H. annuus* the methanol extract of *Helianthus annuus* has significantly demonstrated antiulcer activity in animal models and antimicrobial activity against different isolates., thus justifying its wide spread use by local population in the treatment of peptic ulcers and microbial infections. The leaf extract may, therefore, be of value in development of novel agents in the treatment of peptic ulcers and microbial infections. Further studies are required to confirm the exact mechanism underlining the ulcer healing and antimicrobial properties of the extract.

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