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Gas Chromatography-Mass Spectrometry (GC-MS) Profiling and Anti-ulcer Activity of the Aqueous Extract of *Lophira lanceolata* Leaves

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Abstract

In this study, we investigated the anti- ulcer activity of the aqueous extract of *Lophira lanceolata* Leaves (LLAE) in albino rats using indomethacin- induced ulcer model. Preliminary phytochemical analysis and acute toxicity study were carried out on the extract using standard methods. GC- MS analysis was also performed on LLAE with a view of identifying the bioactive compounds. Preliminary phytochemical analysis of the extract revealed the presence of phenols, flavonoids, alkaloids, saponins, tannins and steroids. The acute toxicity study shows that the extract is safe up to a dose of 5000 mg/kg p.o. Results of the study also showed that LLAE evoked a dose-dependent gastroprotective activity as demonstrated by significant ($P< 0.05$) inhibition of the formation of ulcers induced by indomethacin. GC-MS analysis of the extract revealed the presence of a number of bioactive compounds. It was concluded that LLAE possess anti-ulcer effect possibly mediated by the phytochemicals detected. Thus, LLAE can be used as an alternative to orthodox anti- ulcer drugs or used as an add-on therapy.

Keywords: Anti-ulcer, GC-MS Profiling, Aqueous, *Lophira lanceolata*, Indomethacin

Introduction

Peptic ulcers develop due to excessive secretion of acid and pepsin, a diminished mucosal defence or a combination of these 2 abnormalities. Predisposing factors of gastric ulcer include *Helicobacter pylori* infection, Nonsteroidal anti-inflammatory drugs, cigarette smoking, stress, alcohol and chronic pancreatitis (Tariq *et al.*, 1986). Symptoms of peptic ulcer disease include epigastric pain of a burning or gnawing nature (postprandial pain and pain relieved by food or antacids), nausea, vomiting, belching and bloating. Complications of protracted untreated cases include anaemia caused by Gastro-intestinal blood loss, weight loss attributed to a reduced appetite caused by fear of pain and vomiting associated with a

gastric ulcer or pyloric stenosis and mucosal perforation (Hunt *et al.,* 2006). Although there is a large arsenal of drugs with antiulcerogenic activity on the market, none produces 100% remission of ulcers, with reduced side effects and without compromising the patient's wellbeing, which usually results in chronic use of these drugs. There is, therefore, the need to develop safe, effective and affordable alternatives in the symptomatic management of peptic ulcer disease.

Lophira lanceolata is a tree of the tropical and sub-tropical regions. It is a common tree in Cameroun, Nigeria and Sudan. It often grows gregariously on fallow land at the edge of forests. It is a tree of 8 to 10 m tall, straight or twisted, with leaves alternate, clustered at the end of short straight branches, glabrous, bright and blade oblong-lanceolate. The bark surface is corky grey (Arbonier, 2000). *Lophira lanceolata* is used in traditional medicine to treat several illnesses. The decoction of the fresh leaves is administered orally against headaches, dysentery, diarrhoea, cough, abdominal pains and cardiovascular diseases. It is also used on skin to cure wounds (Arbonier, 2000). The aim of this study was to investigate the anti-ulcer activity of the aqueous extract of *Lophira lanceolata* Leaves and also carry-out a GCMS profiling with a view of identifying the bioactive compounds present in the extract.

Materials and Methods

Materials

Chemicals and drugs

All chemicals used in this study were of analytical grade and were purchased from Sigma Chemical Co. Ltd (USA) through a local vendor. Cimetidine and Indomethacin were purchased from a local pharmacy shop.

Animals

Male adult wistar rats weighing 150–200g were used for this study. They were kept in stainless steel cages under standard laboratory conditions. They were maintained on clean water and standard rodent feed.

Methods

Plant Collection and Identification

The leaves of *Lophira lanceolata* were collected from a natural habitat in Agbeji Area of Kogi State, Nigeria. The plants were identified at the Department of Plant Science and Biotechnology, Kogi State University, Anyigba by Mr. Momoh.

Figure 1: *Lophira lanceolata* in its Natural Habitat

Preparation of Extract

The leaves of the plant were shade- dried for seven (7) days and pulverized using a laboratory mortar and pestle. One thousand and five hundred (1500) gram of the pulverized leaves was soaked in distilled water for 72 h. The resulting mixture was filtered using Whatmann filter paper (Size No1) and the extract was concentrated using a free- dryer. The extract was code-named 'LLAE' and stored in the refrigerator.

Preliminary Phytochemical Analysis of Extract

The extract was analysed for its phytochemical composition using the method of Harborne, (1998).

Acute Toxicity Study

The oral median lethal dose (LD50) of the polyherbal formulations was determined in rats according to the method of Lorke (1983).

Evaluation of antiulcer activity: Indomethacin-induced ulceration

Male adult albino rats were used for the experiment. They were randomly divided into 4 groups of 5 rats each. Food was withdrawn 24 h and water 2 h before the commencement of the experiment (Alphin and Ward, 1967). Group 1 (control) received only indomethacin (Sigma, 60 mg/kg p.o. dissolved in 5% Na_2CO_3); Groups 2 and 3 were pretreated with 200 and 400 mg/ kg p.o of LLAE respectively. Group 4 received cimetidine (100 mg/kg p.o. dissolved in 50% Tween 80). One hour later, groups 2 and 3 were administered indomethacin. Four hours after indomethacin administration, animals were killed by cervical dislocation. The stomachs were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline. Macroscopic examination was carried out with a hand lens and the presence of ulcer lesion was scored (Nwafor *et al.*, 1996). Ulcer index (UI) and preventive ratio (PR) of each of the groups pretreated with extract was calculated using standard methods (Zaidi and Mukerji, 1985; Nwafor *et al.*, 2000).)

GC-MS Analysis

Chemical composition of the fractions was determined by GC/MS-QP-2010 plus Ultra (Shimadzu, Kyoto Japan).

Statistical Analysis

Statistical analysis was carried out using SPSS version 20.0. All the data were expressed as mean ± SEM and the statistical differences between the means were determined by one way analysis of variance (ANOVA) which was followed by Turkey-Kramer multiple comparison and difference between means at $P > 0.05$ were considered significant.

Results

Phytochemical Composition of the Aqueous Extract of Lophira lanceolata Leaves (LLAE)

Phytochemical analysis of LLAE revealed the presence of phenols, terpenes, saponins, steroids, glycosides, flavonoids, tannins and alkaloids in varying proportions.

Table 1: Phytochemical composition of the Aqueous Extract of *Lophira lanceolata* Leaves (LLAE)

Phytochemicals	LLAE (Bioavailability)
Phenols	+++
Tannins	++
Saponins	++
Alkaloids	++
Glycosides	+
Flavonoids	++
Terpenoids	+++
Steroids	++

Key: + (slightly present), ++ (moderately present), +++ (highly present)

Acute Toxicity Study

The results of acute toxicity studies showed no sign of toxicity or mortality up to a dose of 5000 mg/kg of the LLAE (Table 1). The oral LD_{50} of the extract was then taken to be > 5000 mg/kg.

Table 2: Observed Effects of Aqueous Extract of *Lophira lanceolata* Leaves (LLAE) on Rats

Phase	Group	Treatment (mg/kg)	D/T	Observed Sign of Toxicity
I	1	LLAE (10)	0/3	-
	2	LLAE (100)	0/3	-
	3	LLAE (1000)	0/3	-
II	1	LLAE (1600)	0/1	-
	2	LLAE (2900)	0/1	-
	3	LLAE (5000)	0/1	-

Key: D= Number of deaths, T= Number of treated animals

Indomethacin-induced Gastric Ulceration

Table 3 shows that indomethacin induced gastric ulcer in all experimental groups. The extract at 200 and 400 mg/kg had significant reduction ($p < 0.05$) in the gastric erosions formed compared to control as evident in the reduction of ulcer indices. The potency of the extract in reducing ulcer was dose- dependent. LLAE at 200 and 400 mg/kg produced 65.81 and 93.82% inhibition of ulcer respectively. The ulcer inhibition produced by the highest dose of LLAE was comparable to that of cimetidine, the standard anti-ulcer drug used, which produced 95.03% inhibition.

Table 3: Effect of Aqueous Extract of *Lophira lanceolata* Leaves (LLAE) on Indomethacin-induced Gastric Ulcer

Treatment (mg/kg)	Ulcer Index	% Ulcer Inhibition
Control (Indomethacin 60 mg/kg)	15.88±1.23	-
LLAE (200 mg/kg)	5.43±1.01*	65.81
LLAE (400 mg/kg)	0.98±0.56*	93.82
Cimetidine(100 mg/kg)	0.79±0.22*	95.03

Data were expressed as mean ± SEM. significant at *P < 0.05 when compared to control n = 5.

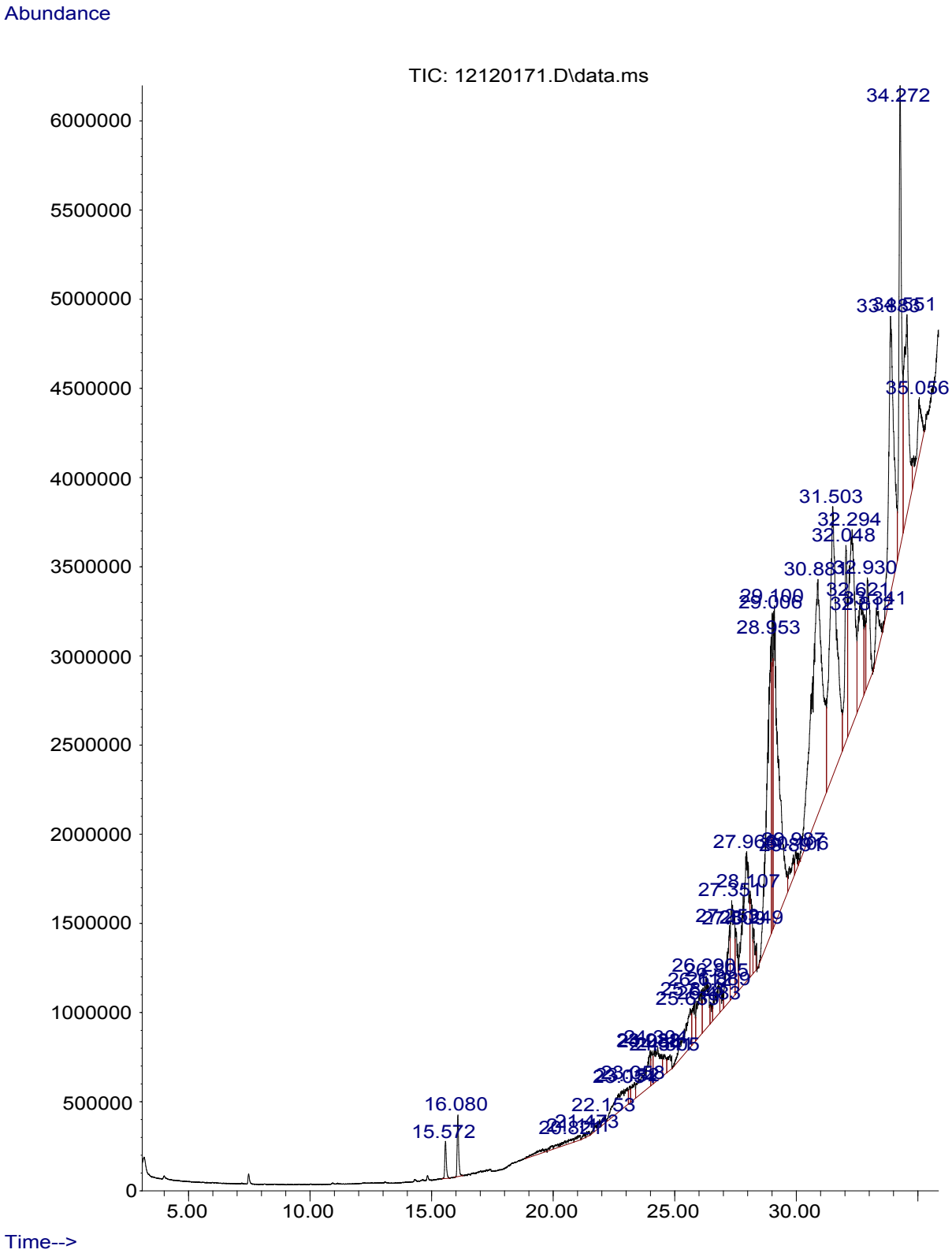

Figure 2: GC-MS Chromatograph of Aqueous Extract of *Lophira lanceolata* Leaves (LLAE)

Table 4: Peak Report TIC

Peak#	R. Time	Area	Name
1	15.5721	0.3071	2,4-Decadienal, (E,E)-
2	16.0805	0.494	2,4-Decadienal, (E,E)-
3	20.8213	0.5487	6-Octadecenoic acid
4	21.1109	0.1108	Octadec-9-enoic acid
5	21.4732	0.1088	9-Octadecenoic acid, (E)-
6	22.1528	0.2862	9-Octadecenoic acid, (E)-
7	23.0541	1.2876	cis-13-Octadecenoic acid
8	23.1516	0.1499	Oleic Acid
9	23.3578	0.3266	Oleic Acid
10	23.9882	1.2921	cis-13-Octadecenoic acid
11	24.0375	0.3202	cis-13-Octadecenoic acid
12	24.3038	1.1251	cis-13-Octadecenoic acid
13	24.5414	0.3399	6-Octadecenoic acid
14	24.8051	0.3077	cis-Vaccenic acid
15	25.6389	1.5401	Oleic Acid
16	25.8277	0.6195	cis-13-Octadecenoic acid
17	26.1123	1.0975	cis-9-Hexadecenal
18	26.2901	1.3849	cis-Vaccenic acid
19	26.4829	0.1995	cis-13-Octadecenoic acid
20	26.8047	0.7038	cis-Vaccenic acid
21	26.8886	0.2714	Oleic Acid
22	27.2527	1.2642	cis-13-Octadecenoic acid
23	27.3513	1.8098	Oleic Acid
24	27.5094	0.7705	cis-Vaccenic acid
25	27.9659	4.1039	cis-Vaccenic acid
26	28.1069	0.9687	1,2-Benzisothiazole, 3-(hexahydro-1H-azepin-1-yl)-, 1,1-dioxide
27	28.2493	0.3953	cis-13-Octadecenoic acid
28	28.9532	6.6965	Cyclopentadecanone, 2-hydroxy-
29	29.0064	2.2694	Ethyl Oleate
30	29.0997	8.795	cis-9-Hexadecenal
31	29.8906	0.4974	6-Octadecenoic acid
32	29.9869	0.2241	cis-9-Hexadecenal
33	30.1061	0.0436	6-Octadecenoic acid
34	30.8807	13.8146	Cyclopentadecanone, 2-hydroxy-
35	31.5031	10.2059	Oleic Acid
36	32.0484	2.6469	2,3-Dihydroxypropyl elaidate
37	32.2944	6.188	13-Tetradecen-1-ol acetate
38	32.621	2.8371	13-Tetradecen-1-ol acetate
39	32.8123	0.6035	2,3-Dihydroxypropyl elaidate
40	32.9304	1.9107	2,3-Dihydroxypropyl elaidate
41	33.3407	0.8897	6-Octadecenoic acid, (Z)-
42	33.8828	7.3962	7-Pentadecyne
43	34.272	6.4648	(R)-(-)-14-Methyl-8-hexadecyn-1-ol
44	34.5509	5.0087	cis-9-Hexadecenal
45	35.0555	1.374	2,3-Dihydroxypropyl elaidate

Discussion

In this study we evaluated the anti-ulcer activity of the aqueous extract of *Lophira lanceolata* on indomethacin ulcer models and we also carried out GC-MS profiling with a view of revealing the phytochemical composition of the extract.

Preliminary phytochemical analysis revealed the presence of phenols, saponins, flavonoids, steroids, glycosides and alkaloids. The acute toxicity studies carried out on the extract revealed no mortality or physical changes in skin and fur, eyes and mucus membrane, respiratory rate, circulatory signs, autonomic and central nervous system effects up to a dose of 5000 mg/kg. The oral LD_{50} of the extract was then taken to be > 5000 mg/kg for. Judging from this study, the extract of *Lophira lanceolata* could be considered to be relatively safe.

In this study, indomethacin was used to induce ulcer in rats. Indomethacin is known to cause ulcer especially in an empty stomach (Bhargava *et al*., 1973) and mostly on the glandular (mucosal) part of the stomach (Evbuonwa and Bolarinwa, 1990; Nwafor *et al*., 1996) by inhibiting prostaglandin synthetase through the cycloxygenase pathway (Rainsford, 1987). Prostaglandins function to protect the stomach from injury by stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow and regulating mucosal turn over and repair (Hayllar and Bjarnason, 1995; Hiruma-Lima *et al*., 2006). This suppression of prostaglandins synthesis by indomethacin results in increased susceptibility of the stomach to mucosal injury and gastro-duodenal ulceration. The extract was observed to significantly and dose- dependently reduce mucosal damage in the indomethacin– induced ulcer model, suggesting the possible extract mobilization and involvement of prostaglandin in the anti- ulcer effect of the extract.
Preliminary phytochemical and GC- MS analyses of the extract revealed the presence of phytochemicals. Some of which might have been responsible for its observed anti- ulcer activity. Flavonoids have been reported to protect the gastric mucosa from damage by increasing the mucosal prostaglandin content and by inhibiting histamine secretion from mast cells by inhibition of histidine decarboxylase. Free radical scavenging ability of flavonoids has been reported to protect the gastrointestinal tract from ulcerative and erosion lesion (Borrelli and Izzo, 2000). Saponins, especially triterpenes type have been implicated in antiulcer activity mediated by formation of protective mucus on the gastric mucosa and also protect the mucosa from acid effects by selectively inhibiting PGF2α (Agwu and Okunji, 1986; Lewis and Hanson, 1991). These phytochemicals therefore must have been responsible for the anti-ulcer potentials of the extract.

Conclusion

In conclusion, the reported results have validated the use of *L. lanceolata* leaves in the therapy of gastric ulcer disease. Hence, it's possible use as an alternative to the orthodox anti- ulcer drugs or as an add-on therapy.

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