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SCREENING OF Cassia sieberiana (FABACEAE) LEAF EXTRACT FOR IN-VITRO ANTI MICROBIAL AND ANTI-ULCER ACTIVITIES

Khan Muluh Emmanuel*, Ugbede, Odokpe Agustine, Tor-Anyiin Terumon Amon

Department of Chemistry, College of Science, university of Agriculture, Makurdi Nigeria

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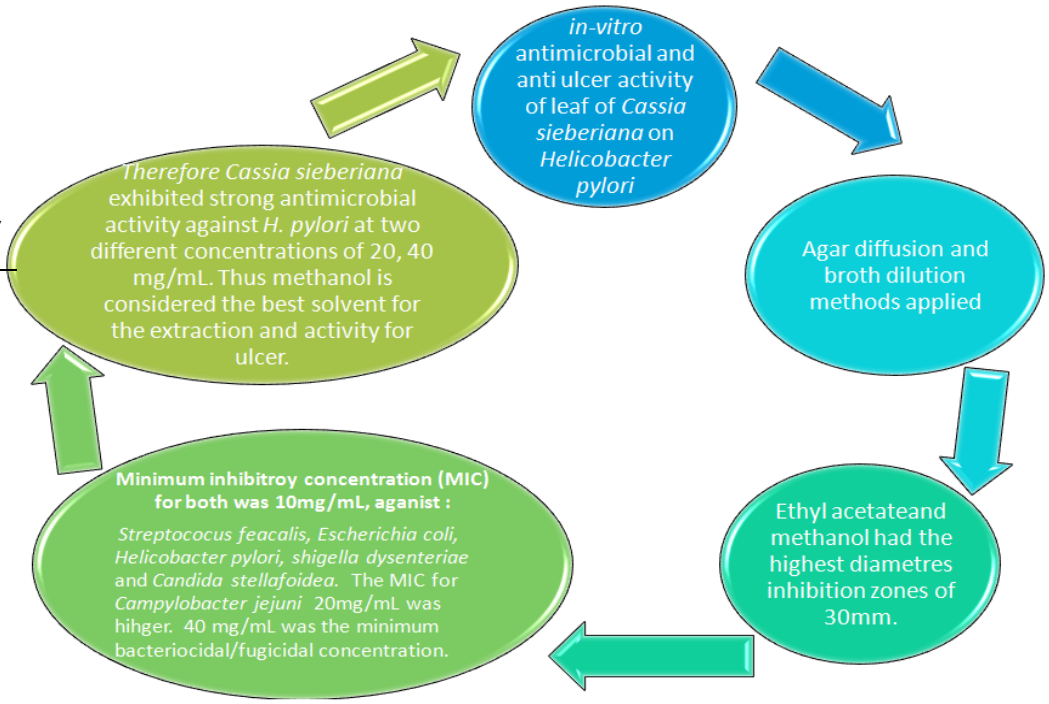
GRAPHICAL ABSTRACT



KEYWORDS

In-vitro
Anti-microbial
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ABSTRACT

Study evaluates the *in-vitro* anti-microbial and anti-ulcer activity of *Cassia sieberiana* leaf extracts on *Helicobacter pylori* the major etiological agent of chronic active gastritis and peptic ulcer disease also called gastric carcinoma. Crude extracts of *cassia sieberiana* were screened for their antimicrobial and antiulcer activities. Antimicrobial assay and in-vitro anti-ulcer (*H. pylori*) was performed using Agar Diffusion Method and Broth Dilution Method. Ethyl acetate extract had the highest diameter of zones of inhibition (30 mm). Ethyl acetate and methanol extracts had the same Minimum inhibitory concentrations of 10 mg/mL against *Streptococus feacalis, Escherichia coli, Helicobacter pylori, shigella dysenteriae* and *Candida stellafoidea* but for *Campylobacter jejuni* which had a minimum inhibitory concentration at 20 mg/mL for methanol extract. Methanol extract had the best minimum bactericidal/fungicidal concentration (40 mg/mL) on the pathogens. The results showed that *Cassia sieberiana* exhibited strong antimicrobial activity against *H. pylori* at two different concentrations of 20, 40 mg/mL.

* Corresponding author: Khan Muluh Emmanuel
Tel/Fax: +2347031667488, E-mail: khandora2000@gmail.com
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1. Introduction

Plants are potential sources of antimicrobial compounds and a number of researchers have examined the antimicrobial activities of medicinal plants used in traditional or alternative healthcare systems [1, 2].

Human pathogenic microorganisms have been accounted to develop resistance to commonly vended antibiotic drugs used in therapy [3]. Nature has blessed animals with plenty of herbs and plants which form the major source of traditional medicines used to help in relief from sickness and are still widely used all over the world. Herbal treatment is still used for many health challenges. Herbs are safe, less toxic, economical and a reliable key natural resource of drugs all over the world [4]. Various parts of *Cassia sieberiana* have been used as folk medicine as diuretic, purgative and to treat stomach ache, malaria, ulcer, gonorrhea, diarrhea, tooth ache, vomiting and dysentery. Also in Cote D'ivoire, it is used as intestinal worm expeller, and to treat venereal diseases, sterility, and dysmenorrhea. In Benin Republic, it is used to treat hemorrhoids, bilharzia, leprosy, sleeping sickness, dropsy and blood dysentery[5, 6]. There has been renewed interest in herbal medicine in several parts of the world with a lot of the herbal remedies being integrated into orthodox medical practice [7].

Some African countries: Egypt, Burkina Faso, Ghana, Nigeria, Zambia and South Africa have also made good advances in the area of the use of plants for production of new drugs [2]. Acceptance of traditional medicine as an alternative form of health care and development of microbial resistance to available antibiotics has provided drive to the search for new antimicrobial substances from various sources including medicinal plants [8-10].

*Cassia sieberiana*is known as "West African Laburnum", "African Laburnum", "drumstick tree" (English) and in Nigeria it is called "Marga" (Hausa),"Okpeheka" (Igala) and "Ifo or Aridantooro" (Yoruba). *Cassia Sieberiana* is mainly a shrub broadly distributed in the Southern Sahel and Sudan Savanna from Senegal to Cameroon, Gambia East to Democratic Republic of Congo and Uganda [11]. In Nigeria it is found in the North-West, areas like the forest reserve near Sokoto, Zamfara, and Zurumi. It is also widely distributed in Bauchi, Borno, Yobe, some part of Adamawa States in the North Eastern part of Nigeria and some parts of the North central [11]. It is mainly found in Agodi in Ibadan and Awka near Onitsha in the South – West and South – East respectively [12, 13]. Peptic ulcer is a gastro intestinal situation due to an imbalance between the defensive factors like prostaglandins, bicarbonate secretion, gastric mucus, innate resistance of the mucosal cell and the aggressive factors like *Helicobacter pylori*, pepsin, and acid [14, 15]. Peptic ulcer disease generally happens in the proximal duodenum and stomach. It is also known as "*ulcuspepticum*" which is a conglomerate of heterogeneous conditions which manifest itself as a break in the lining of the gastro intestinal mucosa that is usually acidic and thus very painful [16]. It develops when aggressive factors overcome the defensive factors [14].

The normal stomach mucosa maintains stability between protective and aggressive factors. Some of the main aggressive factors are pepsin, abnormal motility, gastric acid, bile salts, non-steroidal anti-inflammatory drugs (NSAID) and the use of alcohol, as well as infection with microorganisms (*Helicobacter pylori* and others). On the other hand, gastro protective prostaglandin synthesis, mucus secretion, normal tissue microcirculation and bicarbonate production guard against ulcer formation. Although in most cases the etiology of ulcer is unknown yet, it is commonly believed that gastric ulcers are multi-factorial and develop when aggressive factors (infectious agents, exogenous and/or endogenous) overcome mucosal protection mechanisms [17]. *H. pylori* is a human pathogen that causes chronic gastritis, has a role in gastric and duodenal ulcer, is involved in gastric carcinogenesis and the bacteria have been classified as a definite (Class I) carcinogen to humans [18]. This gram negative gastric pathogen is also considered as a possible significant factor in at least a subset of patients with functional dyspepsia [19].

As many as 80% of ulcers are linked with *Helicobacter pylori*, a spiral-shaped bacterium, that lives in the acidic environment of the stomach. This organism weakens the protective coating of the stomach and first part of the intestine and allows destructive digestive juice to eat away at the sensitive lining below[20-23]. Some drugs such as anticholinergic drugs, histamine H_2-receptor antagonists, cytoprotectants, antacids demulcents, anti cholinergics, and irreversible proton pump inhibitors have been used for the treatment of ulceration. On the other hand, prolonged use of these drugs may lead to series of adverse effects such as thrombocytopenia, nephrotoxicity, hepatotoxicity, gynecomastia and impotence [24]. Due to such side effects produced by conventional drugs, there is a pressing need of more effective and safer treatments with fewer side effects, for the treatment of gastro-duodenal ulcers. So herbal

medicines are well thought-out as better alternatives for the treatment of ulcer [25, 26].

2. MATERIALS AND METHODS

Plant Collection and Authentication

Plant specimens were collected from Egume, Dekina Local Government Area of Kogi State in April 2017 and taken for proper identification at the Forestry Department Federal University of Agriculture Makurdi by a Botanist, Mark Uleh and given a herbarium number UAM/FH/0213. The plant samples were shade dried at room temperature and powdered with the aid of pestle and mortar into coarse powder. It was stored in air tight container until required for the experiment

Sequential Extraction

The powdered leaf material (500g) of *Cassia sieberiana* was extracted with 400 mL of N-Hexane, Ethyl Acetate and Methanol. The extracts were filtered using Whatman N°1 filter paper and the filtrate was then concentrated under reduced pressure using rotary evaporator at 37^0C (70^0C for methanol) and evaporated to constant weight before subsequent analyses [27].

In vitro Antimicrobial Screening

Antimicrobial activities of the plant extracts were carried out using the following pathogens (clinical isolates): *Methicillin Resistant Staphylococcus aureus, Staphylococcus faecalis, Escherichia coli, Campylobacter jejuni, Vibro cholereae, Helicobacter pylori ,shigella dysenteriae, Candida albicans, Candida krusei, Candida stellafoidea* and *Aspergillus sp*. 0.4g of the extract was weighed and dissolved in 10mL Dimethyl sulphoxide (DMSO) to obtain a concentration of 40mg/mL. This served as initial concentration of extracts used to determine antimicrobial activities.

Mueller Hinton and Sabourand Dextrose Agar were the growth media used for bacteria and fungi, respectively. All media were prepared according to manufacturer's instructions, sterilized at 121°C in an autoclave for 15 minutes, poured into sterile petri-dishes and allowed to cool and solidify. Diffusion method was used for screening the extracts. Mueller Hinton Agar was seeded with standard inoculum (0.1 mL) of bacteria and Sabourand Dextrose Agar with the fungi. The inocula were spread evenly over the surface of the media with sterile swabs. A standard cork borer (6 mm in diameter) was used to cut a well at the center of each inoculated medium. Solutions of extracts (0.1 mL each) of concentration 40 mg/mL were then introduced into each well on the medium. Inoculated plates were incubated at 37°C for 24 hours for the bacteria and at 30°C for 7 days for the fungi, after which each plate was observed for inhibition zone of growth. Zones were measured with a transparent ruler and the results recorded in millimeters [28]. The minimum inhibition concentration (MIC) of extracts was carried out using broth dilution method. Mueller Hinton and Sabourand Dextrose Broth were prepared; the broth (10 mL) was dispensed into test tubes and sterilized at 121°C for 15 minutes, then allowed to cool. McFarland's turbidity standard scale number 0.5 was prepared to give a turbid solution. Normal saline (10 mL) was dispensed into sterile test tubes and the test microbes inoculated and incubated at 37°C for 24 hours. Dilution of the test microbes in the normal saline was done until the turbidity matched that of the McFarland's scale by visual comparison at which point, the test microbes had a concentration of about 1.5 x 108 CFU/mL.

A two-fold serial dilution of the extracts in the sterilized broth was made to obtain the concentrations of 40, 20, 10, 5 and 2.5 mg/mL. Initial concentrations were obtained by dissolving extracts (0.4 g) in sterile broth (10 mL). Having obtained the different concentrations of the extracts in the sterile broth, the test microbes in the normal saline (10 mL) were then inoculated into the different concentrations. Incubation was made at 37°C for 7 days for the fungi and 37°C for 24 hours for bacteria, after which the broth was observed for turbidity. Lowest concentration of extracts in the broth which showed no turbidity was recorded as the MIC. Minimum bactericidal concentration/ Minimum fungicidal concentration (MBC/MFC) were carried out to determine whether the test microbes were killed or only their growth was inhibited. Mueller Hinton and Sabourand Dextrose Agar were prepared and sterilized at 121°C for 15 minutes then poured into sterile petri-dishes and allowed to cool and solidify. The content of the MIC in the serial dilution were sub-cultured into the prepared media, incubation were made at 37°C for 24 hours for bacteria and at 30°C for 7 days for fungi, after which plates were observed for colony growth. MBC/MFC were the plates with lowest concentration of extract without colony growth [2].

In-vitro Anti-ulcer Test

As reported by [18], anti-ulcer activities were carried out by determining the minimum inhibition concentration of the leaf extracts. Clinical isolate of *H.pylori* were used to determine the anti-ulcer. Minimum inhibition concentration (MIC) determination was used for the detection of anti-*H. pylori* activity in *Cassia sieberiana*.

This study was designed to evaluate the MICs of *Cassia sieberiana* along with common antibiotics like Ciprofloxacin (bacteria) and Fluconazole (fungi) against *H. pylori* isolates [18].

The minimum inhibition concentration (MIC) of extracts was carried out using broth dilution method. Mueller Hinton and Sabourand Dextrose Broth were prepared; the broth (10 mL) was dispensed into test tubes and sterilized at 121°C for 15 minutes, then allowed to cool. McFarland's turbidity standard scale number 0.5 was prepared to give a turbid solution. Normal saline (10 mL) was dispensed into sterile test tubes and the test microbes inoculated and incubated at 37°C for 24 hours. Dilution of the test microbes in the normal saline was done until the turbidity matched that of the McFarland's scale by visual comparison at which point, the test microbes had a concentration of about 1.5 x 108 CFU/mL. (CFU is colony formation unit).

A two-fold serial dilution of the extracts in the sterilized broth was made to obtain the concentrations of 40, 20, 10, 5 and 2.5 mg/mL. Initial concentrations were obtained by dissolving extracts (0.4 g) in sterile broth (10 mL). Having obtained the different concentrations of the extracts in the sterile broth, the test microbes in the normal saline (10 mL) were then inoculated into the different concentrations. Incubation was made at 37°C for 7 days for the fungi and 37°C for 24 hours for bacteria, after which the broth was observed for turbidity. Lowest concentration of extracts in the broth which showed no turbidity was recorded as the MIC.

3. RESULTS AND DISCUSSION

Antimicrobial Screening

The parameters determined were the zone of inhibition (ZI), minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC). The antimicrobial results obtained revealed that ethyl acetate extract had the highest diameter of zones of inhibition (30 mm) against test microbes with values of 26, 28, 25, 30, 28 and 26mm compared to the standard drug which is 35, 38, -, 34, 38 and - for *Streptococcus faecalis, Escherichia coli, Campylobacter jejuni, Helicobacter pylori, Shigella dysenteriae* and *Candida stellafoidea* respectively followed by methanol extract: 23, 25, 21, 24, 26 and 22 in the same order. The hexane extract had the smallest diameters of zone of inhibition. Ethyl acetate and methanol extracts had the same Minimum inhibitory concentrations of 10 mg/mL against *Streptococus feacalis, Escherichia coli, Helicobacter pylori, shigella dysenteriae* and *Candida stellafoidea.* But for *Campylobacter jejuni* which had a minimum inhibitory concentration at 20mg/mL for methanol extract. Methanol extract had the best minimum bactericidal/fungicidal concentration (40 mg/mL) but for *Streptococcus faecalis* at 20 mg/mL.*Methicillin resistant staphylococcus aureas, Vitro cholereare, Candida albicans*, and *Aspergillus sp* were resistant to all extract (Table 1). Six of the organisms showed sensitivity to the extracts as shown in Table 1.

Table 1: The Antimicrobial Screening of *Cassia Sieberiana* leaf extracts.

Organism	HE	EA	ME	CF	FZ
MRSA	R	R	R	R	R
S. faecalis	S	S	S	S	R
E. coli	S	S	S	S	R
C. jejuni	S	S	S	S	R
V. cholereae	R	R	R	R	R
H. pylori	S	S	S	S	R
S. dysenteriae	S	S	S	S	R
C. albicans	R	R	R	R	S
C. krusei	R	R	R	R	S
C. stellafoidea	S	S	S	S	S
Aspergillus Sp	R	R	R	R	R

KEY: HE = Hexane, EA = Ethyl acetate, ME = Methanol, CF = Ciprofloxacin, FZ = Fluconazole, S = Sensitive, R = Resistance, MRSA = Methicillin Resistant Staphylococcus aureus

Ethyl acetate extract showed the widest zones of inhibition against *Helicobacter pylori* at 30 mm and 28 mm for both *Escherichia* coli and *Shigella dysenteriae* respectively (Table 2), with minimum inhibition concentration (MIC) of 10 mg/mL and minimum bacteridical concentration (MBC) of 20 mg/mL on *Helicobacter pylori*.

Table 2: Zone of Inhibition (mm) of Extract against the test Microorganisms

Test Organism	EA	HE	ME	CF	FZ
MRSA	-	-	-	-	-
S. faecalis	26	20	23	35	-
E. coli	28	20	25	38	-
C. jejuni	25	18	21	-	-
V. cholerea	-	-	-	34	-
H. pylori	30	21	24	35	-
S. dysenteriae	28	20	26	38	-
C. albicans	-	-	-	-	35
C. krusei	-	-	-	-	31
C. stellafoidea	26	20	22	-	32
Aspergillus Sp	-	-	-	-	-

KEY: - = No Inhibition

Table 3: Minimum Inhibition Concentration (MIC) of the Extract against the Test Microorganisms

Test Organism	EA					HE					ME				
	40 mg/ml	20 mg/ml	10 mg/ml	5.0 mg/ml	2.5 mg/ml	40 mg/ml	20 mg/ml	10 mg/ml	5.0 mg/ml	2.5 mg/ml	40 mg/ml	20 mg/ml	10 mg/ml	5.0 mg/ml	2.5 mg/ml
MRSA	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
S. faecalis	-	-	µ	+	++	-	-	µ	+	++	-	-	µ	+	++
E. coli	-	-	µ	+	++	-	-	-	µ	+	-	-	µ	+	++
C. jejuni	-	-	µ	+	++	-	-	µ	+	++	-	µ	+	++	+++
V. cholereae	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
H. pylori	-	-	µ	+	++	-	-	-	µ	+	-	-	µ	+	++
S. dysenteriae	-	-	µ	+	++	-	-	-	µ	+	-	-	µ	+	++
C. albicans	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
C. krusei	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
C. stellafoidea	-	-	µ	+	++	-	-	µ	+	++	-	-	µ	+	++
Aspergillus Sp	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

Key: - = No Turbidity (No growth), µ = MIC, + = Turbidity (Low growth), ++ = Moderate Turbidity, +++ = Heavy Turbidity, R = Pathogen is Resistant to Extract, MRSA = Methicillin Resistant Staphylococcus aureus

Table 4: Minimum Bactericidal/Fungicidal Concentration of the Extract Against the test Microorganisms

Test Organism	EA					HE					ME				
	40 mg/ml	20 mg/ml	10 mg/ml	5.0 mg/ml	2.5 mg/ml	40 mg/ml	20 mg/ml	10 mg/ml	5.0 mg/ml	2.5 mg/ml	40 mg/ml	20 mg/ml	10 mg/ml	5.0 mg/ml	2.5 mg/ml
MRSA	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
S. faecalis	-	µ	+	++	+++	-	µ	+	++	+++	-	µ	+	++	+++
E. coli	-	µ	+	++	+++	-	-	µ	+	++	µ	+	++	+++	+++
C. jejuni	µ	+	++	+++	++++	-	µ	+	++	+++	µ	+	++	+++	+++
V. cholereae	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
H. pylori	-	µ	+	++	+++	-	-	µ	+	++	µ	+	++	+++	++++
S. dysenteriae	-	µ	+	++	+++	-	-	µ	+	++	µ	+	++	+++	+++
C. albicans	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
C. krusei	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
C. stellafoidea	µ	+	++	+++	++++	-	µ	+	++	+++	µ	+	++	+++	++++
Aspergillus Sp	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

KEY: - = No Colony growth, µ = MBC/MFC, + = Scanty Colonies Growth, ++ = Moderate Colonies growth, +++ = High Colonies Growth, ++++ = Heavy Colonies Growth.

The hexane extract showed a minimum inhibition concentration (MIC) of (10 mg/mL) for *Streptococcus faecalis, Campylobacter jejuni* and *Candida stellafoidea* respectively and (5.0 mg/mL) for *Escherichia coli, Helicobacter pylori* and *Shigella dysenteriae* respectively (Table 3). Tested pathogens showed MBC/MFC activity at 40 mg/mL except hexane extract that was not active against any of the pathogens at 40 mg/mL (Table 4). Leaf extracts from *Cassia sieberiana* reportedly have broad spectrum antibacterial and antifungal activities against tested pathogens.

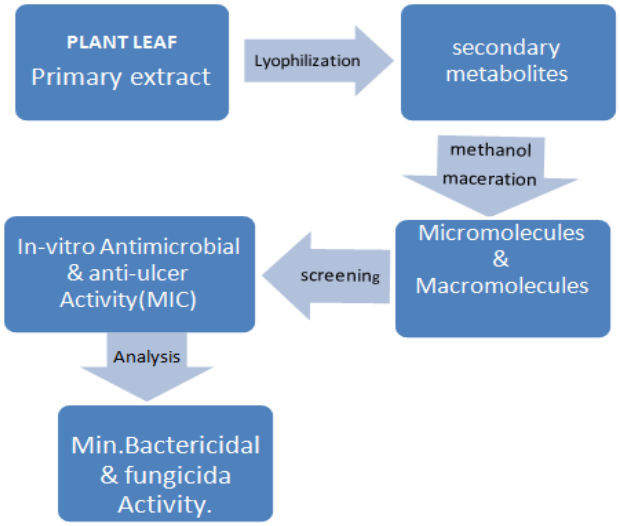

Fig 1: Preparative steps in the leaf extraction

In-vitro anti-ulcer (anti *Helicobacter pylori*)

Tables 5 and 6 showed the zones of inhibition MIC and values of *C. sieberiana* at different concentrations on *H. pylori*. From the results, there is low turbidity at concentrations 5.0 mg/mL for ethyl acetate and methanol extracts, and also they both have moderate responses or turbidity at 2.5 mg/mL concentration of the extract. The inhibition zone of the extract against *Helicobacter pylori* increased with increase in the concentration of the extract. The Minimum inhibition zone of the extract against *Helicobacter Pylori* for both ethyl acetate and methanol extracts is at 10 mg/mL. Thus it is clear from Table 6, that at concentrations 20 and 40 mg/mL of the extracts, there is no growth of *Helicobacter pylori* indicating its total inhibition. Ciprofloxacin used as a reference antibiotic showed inhibitory activity against *H. pylori* while Fluconazole (FZ) as a negative control did not. Peptic ulcer disease caused by *H. pylori* infection is a worldwide problem and the cost of getting rid of it using standard antibiotic regimen is high. Consequently exploration of alternate treatment based on herbal medicine would not only provide major boost to fight multi-drug resistant *H. pylori* but will also provide a simple, inexpensive means to resolve this global health hazard [18]. To combat this antibiotic resistant by *H. pylori*, several medicinal plants are been increasingly used owing to their antibacterial properties. There are many studies both *in vitro* and *in vivo* which have effectively demonstrated antibacterial activities viz; ginger, thyme, evodia, berberine, and curcumin extracts [29]. However there are only few studies which have demonstrated antibacterial activity of *Cassia sieberiana* extracts against *H. pylori* infection. This study shows that *C. sieberiana* leaf extract is active against *H. pylori* and a cure for ulcer.

Table 5: Zone of inhibition (mm) of the *C. sieberiana* extracts on *Helicobacter pylori*

	EA	HE	ME	CF	FZ
H. Pylori	30	21	24	35	0

KEY: CF= Ciprofloxacin, FZ= Fluconazole, EA= ethylacetate extract, HE= hexane extract, ME= methanol extract

Table 6: Data showing zone of minimum inhibition concentration (MIC) of the extract against *Helicobacter pylori*

	Extract		
Concentration	**EA**	**HE**	**ME**
40 mg/ml	-	-	-
20 mg/ml	-	-	-
10 mg/ml	µ	-	µ
5.0 mg/ml	+	µ	+
2.5 mg/ml	++	+	++

KEY: - = No turbidity (No growth), µ = MIC, + = Turbidity (Low growth), ++ = Moderate Turbidity.

CONCLUSION

The present study represents the first directed work to test exclusively the *in vitro* activity against *Helicobacter pylori* of *Cassia sieberiena* leaf extracts. This report shows a strong anti *Helicobacter pylori* activity of *Cassia sieberiana* leaf extracts . Our results provide important information about the anti-*Helicobacter pylori (*anti -ulcer*)* activity of *Cassia sieberiana* Leaf extracts, which will become the starting material for bioassay guided fractionation to determine the active constituents of the plant extracts. These data also contribute to the understanding of the mode of action of the plant extract and to the development of new anti-ulcer therapies. Like anti-ulcer drugs, it believed that the active component(s) of *C. sieberiana* are non-competitive or allosteric inhibitors that bind to a different region of the enzyme and are therefore not competing with the substrate for the active site. Since moulding takes place, there is bound to be a change in shape which hides the active site of the enzyme and thus the active site can no long fit / react.

RECOMMENDATION

It is recommended that an *in vivo* investigation be carried out on the extract using animal models, followed by human clinical trials to provide more conclusive evidence of their safety and efficacy before marketing.

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COMPETING INTERESTS

Authors, *M.E. Khan, M. Austen,* and *T.A. Tor-Anyiin* have declared that no competing interests exist.

References

[1] F. Hamill, S. Apio, N. Mubiru, R. Bukenya-Ziraba, M. Mosango, O. Maganyi and D. Soejarto, Traditional herbal drugs of Southern Uganda, II: literature analysis and antimicrobial assays. *Journal of Ethnopharmacology*, 84 (2003) 57-78.

[2] Y. Dikko, M. Khan, T. Tor-Anyiin, J. Anyam and U. Linus, In vitro Antimicrobial Activity of Fruit Pulp Extracts of Azanza garckeana (F. Hoffm.) Exell & Hillc. and Isolation of One of its Active Principles, Betulinic Acid. *Journal of Pharmaceutical Research International*, (2016) 1-10.

[3] A. GOA, S. Chollom, J. Gotep, N. Gambo, A. Tyem, I. Okeke, O. Nwankiti and A. Okwori, Evaluation of antimicrobial potential of ethanolic leaf and stem bark extracts of Tamarindus indica. (2012)

[4] S. Al-Daihan, M. Al-Faham, N. Al-shawi, R. Almayman, A. Brnawi and R. shafi Bhat, Antibacterial activity and phytochemical screening of some medicinal plants commonly used in Saudi Arabia against selected pathogenic microorganisms. *Journal of King Saud University-Science*, 25 (2013) 115-120.

[5] D.A. Awomukwu, B.L. Nyananyo, A.I. Ikpeama and C.U. Adieze, Comparative chemical constituents of some Cassia species and their pharmacognistic importance in South Eastern Nigeria. *Science Journal of Chemistry*, 3 (2015) 40-49.

[6] M. Arbonnier, *Trees, shrubs and lianas of West African dry zones*. (2004): Quae.

[7] A. Sofowora, Recent trends in research into African medicinal plants. *Journal of ethnopharmacology*, 38 (1993) 197-208.

[8] L. Buwa and J. Van Staden, Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa. *Journal of ethnopharmacology*, 103 (2006) 139-142.

[9] A. Babaei and A.R. Taheri, Direct electrochemistry and electrocatalysis of myoglobin immobilized on a novel chitosan-nickel hydroxide nanoparticles-carbon nanotubes biocomposite modified glassy carbon electrode. *Anal. Bioanal. Electrochem*, 4 (2012) 342-356.

[10] A. Taheri and S. Mohammadi, Ultrasensitive and selective non-enzymatic glucose detection based on pt electrode modified by carbon nanotubes@ graphene oxide/nickel hydroxide-Nafion hybrid

composite in alkaline media. *Progress in Chemical and Biochemical Research*, 1 (2018) 1-10.
[11] A. Michael, Trees, shrubs and lianas of West Africa dry zones. *Cirad-Margraf Publishers Gmbh-Mnhn, pp573*, (2004)
[12] R. Keay, Trees of Nigeria (A revised edition of Nigeria Trees) Oxford University press. *New York, USA*, (1989) 318-323.
[13] S.E. Atawodi, M.L. Liman, O.D. Olowoniyi and G.O. Adejo, *Some Wild Herbaceous Plants of Nigeria: A Biological, Pharmacognostic and Phytochemical Review*, in *Medicinal and Aromatic Plants of the World-Africa Volume 3*. (2017), Springer. 249-282.
[14] T.L. Srinivas, S.M. Lakshmi, S.N. Shama, G.K. Reddy and K. Prasanna, Medicinal plants as anti-ulcer agents. *Journal of Pharmacognosy and Phytochemistry*, 2 (2013) 91-97.
[15] N. Dashputre and N. Naikwade, Evaluation of anti-ulcer activity of methanolic extract of Abutilon indicum Linn leaves in experimental rats. *Int J Pharm Sci Drug Res*, 3 (2011) 97-100.
[16] D.J.E. Cullen, G.M. Hawkey, D.C. Greenwood, H. Humphreys, V. Shepherd, R.F.A. Logan and C.J. Hawkey, Peptic ulcer bleeding in the elderly: relative roles ofHelicobacter pylori and non-steroidal anti-inflammatory drugs. . *Gut*, 41 (1997) 459-462.
[17] R. Jalilian and A. Taheri, Synthesis and application of a novel core-shell-shell magnetic ion imprinted polymer as a selective adsorbent of trace amounts of silver ions. *e-Polymers*, 18 (2018) 123-134.
[18] C. Kumari, C. Prasad and J.S. Ramulu, Determination of in-vitro and in-vivo activities of Aloe vera L. against H. pylori. *International Journal of Pharma and Bio Sciences*, 1 (2010)
[19] S. Heron and E. Yarnell, A Botanical Protocol for Helicobacter pylori Infection. *Alternative and Complementary Therapies*, 6 (2000) 60-68.
[20] M. Nabati and V. Bodaghi-Namileh, Molecular Modeling of 3-(1,3-Dioxoisoindolin-2-yl)benzyl Nitrate and its Molecular Docking Study with Phosphodiesterase-5 (PDE5). *Advanced Journal of Chemistry, Section A: Theoretical, Engineering and Applied Chemistry*, 3 (2020) 58-69.
[21] H. Aliyan, Friedel-Crafts alkylation of indoles with epoxides using PW12-APTES@SBA-15. *Iranian Chemical Communication*, 6 (2018) 325-333.
[22] M. Nabati, E. Lohrasbi, H. Sabahnoo, V. Bodaghi-Namileh, M. Mazidi, H. Mohammadnejad-Mehrabani, A. Tavakkoli and A. Gervand, In Silico Study of Metoclopramide as A Small Molecule of Dopamine D2 Receptor: a Quantum-Mechanical (QM) Based Molecular Docking Treatment. *Chemical Methodologies*, 4 (2020) 19-33.
[23] I. Abdurrahman, H. Yu-Lai, Y. Cai-Xia and T.E. Hagr, Identification and characterization of diterpenes from ethyl acetate fraction of stem barks of Boswellia papyrifera (del) hochst, sudanese medicinal plant. *Asian Journal of Green Chemistry*, 3 (2019) 322-335.
[24] M. Thabrew and L. Arawwawala, An Overview of In Vivo and In Vitro Models that can be used for Evaluating Anti-Gastric Ulcer Potential of Medicinal Plants. (2018)
[25] E. Sheen and G. Triadafilopoulos, Adverse effects of long-term proton pump inhibitor therapy. *Digestive diseases and sciences*, 56 (2011) 931-950.
[26] P. Mahendran, A. Vanisree and C. Shyamala Devi, The antiulcer activity of Garcinia cambogia extract against indomethacin-induced gastric ulcer in rats. *Phytotherapy research*, 16 (2002) 80-83.
[27] M.K. Aadesariya, V.R. Ram and P.N. Dave, Investigation of phytochemicals in methanolic leaves extracts of Abutilon pannosum and Grewia tenax by Q-TOF LC/MS. *Progress in Chemical and Biochemical Research*, 2 (2019) 13-19.
[28] T. Tor-Anyiin, J. Igoli, J. Anyam and J. Anyam, ISOLATION AND ANTIMICROBIAL ACTIVITY OF SARRACENIN FROM ROOT BARK OF STRYCHNOS SPINOSA. *Journal of Chemical Society of Nigeria*, 40 (2015)
[29] A. Smith-Palmer, J. Stewart and L. Fyfe, Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. *Letters in applied microbiology*, 26 (1998) 118-122.