ANTIMICROBIAL ACTIVITIES OF EXTRACTS OF *SIDA ACUTA* ON *HELICOBACTER PYLORI*

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Abstract

Sida acuta is a shrub found in the tropics. The plant has been ethno - botanically reported to be used in treatment of cancer, tumor, arthritis and fever. This study on *H. pylori* investigates the antimicrobial activities of various plant parts extracts of *Sida acuta*. The leaf (201.7g) and stem (147.9g) were cleaned, shade dried and pulverized and extracted using the Soxhlet method. Water and ethanol were used for the extraction of active ingredients from the dried powdered leaves and stems. Antimicrobial sensitivity testing was carried out at different concentrations (25mg/ml, 50mg/ml, 100mg/ml, 200mg/ml and 400mg/ml) using agar well diffusion method. McFarland method was used to standardize the inoculum (*H. pylori*). The zones of inhibition were measured in millimetres. At 25mg/ml only ASESA and ELESA inhibited the test organism with diameter zones of inhibition of 12mm and 8mm respectively. The highest zone of inhibition was recorded by ASESA and ESESA with zones of inhibition of 12mm. Phytochemical investigations of all the extracts showed the presence of flavonoids and alkaloids. In addition, aqueous stem extract of *Sida acuta* (ASESA) showed the presences of saponins and terpenes; aqueous leaf extract of *Sida acuta* (ALESA) indicated cardiac glycosides; ethanolic stem extract of *Sida acuta* (ESESA) showed the presence of all the tested phytochemicals while ethanolic leaf extracts of *sida acuta* (ELESA) also showed all the tested phytochemicals except terpenes. Hence, *Sida acuta* could also be a new source for antibiotics discovery and infection treatment.

Keywords: *Sida acuta*, Phytochemical, Ethanolic extract, Antimicrobial.

Introduction

Since the beginning of human civilization, medicinal plants have been used by mankind for their nutritional and therapeutic values. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources.

Sida acuta Burm.f (Malvaceae) is one of those plants currently used by indigenous people for the management of some health problems. This plant is an erect, branched small perennial herb or small shrub of about1.5m height(fig.1) (Mohideen *et al.,* 2002).

Fig 1 *sida acuta* plant

According to (Enechi and Nwodo, 2014), Peptic/or gastric ulcer is an inflamed break in the lining of the stomach or the duodenum caused due to either increased acid production or damage to the mucus lining of the stomach. Ulcer is a prevalent disease affecting around 5-10% of the world's population. (Lauret *et al., 2015).* It is a problem of the gastrointestinal tract characterized by mucosa damage as a result of pepsin and gastric acid secretion. (Ramakrishnan and Salinas, 2007). The pathophysiology of Ulcer involves an imbalance between the offensive (acid production, H. pylori and pepsin) and defensive factor (mucin, prostaglandins, bicarbonate and nitric oxide). An ulcer occurs when the acid and pepsin overcome these natural defence and cause a break in the lining. Therefore ''No gastric acid, no Ulcer is a misconception'' (John Hopkins Medcine, 2018). An Ulcer in the stomach is known as gastric ulcer while that in the first part of intestine is known as duodenal ulcer. *Sida acuta* is one of those plants currently used by indigenous people for the management of some health problems.

Peptic ulcer is a common, serious and significant health care occurrence in the young, frail and elderly. Increasing resistance of *H. pylori* to common antibiotic has led to unsuccessful treatment in full eradication of infections and recurrent infections. However, prolonged use of conventional drugs is often associated with adverse reactions such as hypersensitivity, arrhythmia, impotence, gynecomastia as well as hematopoietic disorders among others (Malfertheiner *et al,* 2009). Therefore the purpose of this study is to provide a novel natural healthy and low risk plant materials like *Sida acuta* which contains active gastro-protective components to be evaluated.

Materials And Methods

Ethical Approval

Ethical approval was obtained from the Chukwuemeka Odumegwu Ojukwu Teaching Hospital COOUTH, Awka where faecal samples of 50 ulcer patients were collected.

Plant Materials and Extraction Procedure

The leaves and stems of *Sida acuta* were collected from Ekwulobia, Anambra State, Nigeria and authenticated in the department of Botany Nnamdi Azikiwe University, Awka. The collected plant materials were cleaned, shade dried and powdered. The powdered leaves were divided into two parts of 1009g each. 1.8 litres of ethanol and 1.5 litres of water were used for extraction. The powdered stems were

divided in two parts, 739.5g each. 1.2litres of ethanol and 1.1litres of water was used to concentrate the extract. The coarse powders were extracted with ethanol solvent (90% v/v) in Soxhlet apparatus and Whatman No.1 filter paper.

Table 1. Preparation of Plant Extract

Extracts	Weight of Final Extract	Percentage Yield
Aqueous Leaves Extract of *sida acuta* (ALESA)	15.82g	15.68%
Aqueous Stem Extract of *sida acuta* (ASESA)	12.67g	17.13%
Ethanolic Leaves Extract of *sida acuta* (ELESA)	19.28g	19.11%
Ethanolic Stem Extract of *sida acuta* (ESESA)	13.08g	17.69%

$$\%\ Yield = \frac{Weight\ of\ Extract}{Weight\ of\ Pulverized\ Leaves\ or\ Stem} \times \frac{100}{1}$$

Isolation of *H. Pylori*

One gram of different faecal samples was weighed and dipped into various test tubes containing 9.0ml of phosphate buffered saline (PBS). The test tube was swirled and one loopful of various content from the test tube was aseptically introduced into Columbia agar media with *H . pylori* selective supplement 10% sheep red blood cell. They were incubated under a microaerophillic environment at 37^0 C for 5days (Sudhakar *et al.,* 2008).

Morphological Identification and Characterization of *H. pylori*

H.pylori is Milkish, circular smooth colony with comma and margin on Columbia algar Gram negative, catalase, oxidase and urease positive. spiral/coma, shaped rods.

Qualitative Analysis of *Sida acuta* Phytoconstituents:

Quantitative analysis of *Sida acuta* Phytoconstituents was carried out for cardiac glycosides, Saponins, Tannins, Flavonoids, Alkaloids, Terpenes and Steroids.

Antibacterial Activity of *Sida acuta* Extracts on H. pylori

The tube dilution assay was employed to first determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the ethanolic plant extract. Two-fold dilution of 400 mg of the plant ethanolic extract was made serially in 10% Dimethylsulfoxide, to get 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml. Thereafter, 1 ml aliquot of each diluted extract was transferred into test tubes containing 1 ml peptone water with 0.1 ml 24 h *Helicobacter pylori* culture of 0.5 Mcfarland turbidity. The set-up was incubated for 24 h at room temperature and turbidity was checked for in each tube. The agar well diffusion method was employed to determine the zones of inhibition of the MIC. A 100 µl aliquot of each concentration of plant extract was placed separately in a well cut in sterile Columbia Agar plate that was already seeded with H. pylori. The plates were incubated in an anaerobic chamber at 35^0C for 24 h and the diameters of zone of inhibition were measured.

Result

Table 2: Colony, Biochemical and Characteristics of *H .pylori*

Test Parameters	Results
Colony morphology	Milkish, circular smooth colony with comma and margin on Columbia algar
Gram stain	Gram –ve, spiral/coma shaped rods
Catalase test	+
Oxidase test	+
Urease	+

Table 3: Phytochemical Result (Qualitative) of various plants parts extracts of sida acuta

Phytochemicals	ALESA	ASESA	ELESA	ESESA
Cardiac glycosides	+	—	+	+
Saponins	—	+	+	+
Tannins	—	—	+	+
Flavonoids	+	++	+	++
Alkaloids	+	+	++	+
Terpens	—	+	—	+
Steroids	—	—	+	+

Key : + = present
: — = Absent

- **ALESA** = Aqueous leave extract of *sida acuta*
- **ASESA** = Aqueous stem extract of *sida acuta*
- **ELESA** = Ethanolic leave extract of *sida acuta*
- **ESESA** = Ethanolic stem extract of *sida acuta*

Table 4: Antibacterial Activity of *S. acuta* Extract against *H .pylori* using tube dilution Assay

Conc. (mg/ml)	ALESA	ASESA	ELESA	ESESA
400	+	+	+	+
200	+	+	+	+
100	+	+	+	+
50	+	+	+	+
25	—	+	+	—
12.5	—	—	—	—
6.25	—	—	—	—

Key: **+ = *Inhibition***

- **—** = No Inhibition
- **ALESA** = Aqueous leave extract of *sida acuta*
- **ASESA** = Aqueous stem extract of *sida acuta*
- **ELESA** = Ethanolic leave extract of *sida acuta*
- **ESESA** = Ethanolic stem extract of *sida acuta*

Table 5: Diameter zone of Inhibition of *Sida acuta* Extract MIC

Extracts	MIC (mg/ml)	Diameter zone of Inhibition (mm)
ASESA	25	12
ALESA	50	10
ESESA	50	12
ELESA	25	8

- **ALESA** = Aqueous leave extract of *Sida acuta*
- **ASESA** = Aqueous stem extract of *Sida acuta*
- **ELESA** = Ethanolic leave extract of *Sida acuta*
- **ESESA** = Ethanolic stem extract of *Sida acuta*

Discussion

Medical plants have been remedies for human disease for a long time because they contain components of therapeutic value (Adegoke *et al.,* 2009). Traditionally used medicinal plants produce a variety of compounds with known therapeutic properties. The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered candidates for developing new antimicrobial drugs (Ahmad and Beg, 2001). Medicinal herbs have curative properties due to the presence of various complex chemical substance of different composition, which are found as secondary plant metabolites in one or more parts of these plants (Patil *et al.,* 2009). Today, there is a renewed interest in traditional medicine and increasing demand for more drugs from plant source. This revival of interest in plant derived drug is mainly due to the current widespread belief that "green medicine" is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects (Nair and chanda, 2007). This situation gives researchers the zeal to search for new antimicrobial substances from various sources like medicinal plants (Cordell, 2000).

H.pylori is a Gram negative spiral/comma shaped rod. It has a milkish, circular smooth colony with convex margin on columbia media enriched with defibrinated sheep red blood cell. It is catalase positive, oxidase positive and urease positive (table 2) .

Antibacterial activity of *S. acuta* extract gave a minimum inhibitory concentration of 25mg/ml, 50mg/ml, 50mg/ml and 25mg/ml respectively for aqueos stem

extract, aqueos leaf extract, ethanolic stem extract and ethanolic leaf extract (table 3). The diameter zones of inhibitions are 12mm, 10mm, 12mm and 8mm respectively (table 4). However, 100mg/ml of the extract were used for the invivo studies. The antimicrobial activities according to tube dilution and agar well diffusion assays of various plant extract are shown in table 3 and table 4.it was found that the extracts of plants had antibacterial activity to the microorganism tested (*H . pylori*), (table 4).

S. acuta is a plant of wide usage in traditional medicine. In *traditional* medicine, the plant is often assumed to treat diseases such as fever, head ache, skin diseases, darrhea and dysentery. Following these traditional usages many studies have been conducted in laboratories for the efficiency of the plant, in addition the plant may have many other properties since it has not been tested for all desired pharmacological activities.

Phytochemicals are secondary metabolites of plants known to exhibit diverse pharmacological and biochemical effects on living organisms. Secondary metabolites play a role in the medicinal properties of plants (Abutbul *et al.,* 2005). The phytochemicals, tannins, saponins, alkaloids, flavonoids, terpenes and phenolics were found to be present in *S.acuta* leaves and are in amounts to be of medicinal value. Many plants containing alkaloids and flavonoids have diuretic, antispasmodic, anti-inflammatory and analgesic effects. The anti ulcerogenic and antibacterial activity of *S. acuta* have been demonstrated on a rat model in which ulcer induction was induced by aspirin plus pylorus ligation, aspirin and ethanol treatment (Akilandeswari *et al.*,2010; Wiyada *et al*., 2018).The qualitative analysis of the ethanol and aqueous extracts of the leaves and stem of *sida acuta* was determined. It has been observed in the previous studies that alcoholic solvents, like ethanol and methanol are more suitable than aqueous solvents in extracting components of medicinal plants (Cowan, 1999).

In the present study, the presence of cardiac glycosides, saponins, tannins, flavonoids, alkaloids, terpens and steroids was obtained in ethanolic extracts of leaf and stem of *Sida acuta* (ELESA and ESESA) with the exeption of terpens in etanolic leaf extract of *S .acuta*. Also in aqueous extracts of leaf and stem of *sida acuta* (ALESA and ASESA) both has flavonoids and alkaloids, absence of steroids and tannins while saponins and terpens were absent in ALESA but present in ASESA. Cardiac glycoside was present in ALESA but absent in ASESA, (Table 2). In this phytochemicals analysis both ethanolic and aqueous leaf and stem extracts of *sida acuta* have the presence of flavonoids and alkaloids (Table 2). Similarly other works of people contain other constituents with flavonoids and alkaloids always present (Senthilkumar et al., 2018; Ezeabara and Egenti, 2018). The presence of flavonoids and alkaloids in different plant parts of *sida acuta* extract is a good indication that the plant has active ulcer protective potentials. Similar phytochemical constituents was also reported by Eleazu *et al.* (2011) who opined that most of the flavonoids and alkaloids present in mature unripe plantain were known to contain antioxidant, antimicrobial, antiviral, anticancer and

antiulcer. This is also supported by the study carried out by Bors *et al*. (1990), who reported that flavonoids could be nutritionally helpful by triggering enzymes that reduce the risk of certain diseases such as ulcer, cancers, heart diseases and age related degenerative diseases. These probably suggested that aqueous and ethanolic leaves and stem extract of *Sida acuta* exudates might have the ability to scavenge free radicals due to the presence of flavonoids. The presence of flavonoids and alkaloid content in *Sida acuta* is very favourable which is significantly higher than the unripe plantain (Eleazu *et al*, 2011). Flavonoids are also known to exhibit anti-inflammatory, anti-neoplastic, and hepatoprotective activities (Havsteen, 1983). More recently they have been shown to reduce acid secretion from gastric parietal cells (Beil *et al.*, 1995). Ulcer induction by *H. Pylori* stimulates gastric secretions which results to increased hydrochloric acid secretion and decreased cytoprotectivity of the mucosal barrier, thus the pathology of this experiment on ulcer. (Patnaick *et al*., 1980; Parmar *et al.*, 1987).

Conclusion

The present study reported that ethanolic and aqueous leaf and stem extracts of *Sida acuta* have established a significant antiulcer activity against ulcer causing microorganism *(H. pylori).* However, the microorganism was inhibited by all the extracts, thus Sida acuta could also be a new source of antibiotics discovery and infection treatment. Hence, it is widely accepted that technology is the key driver of economic growth of countries, with this knowledge it can help in revamping the economy of the country in antibiotic production treatment of ulcer patients.

Recommendation

Further studies are entitled to be carried out on the antimicrobial activities of leaf and stem extracts of *sida acuta* on *H.pylori* isolates from ulcer patients to establish its exact mode of action and active principles involved in its antiulcer effect.

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