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Phytochemical Profiling, GC-MS Analysis and Antibacterial Activity of *Sida acuta leave* Extracts against *Helicobacter pylori*

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Abstract

Helicobacter pylori is a major cause of ulcers, and increasing resistance to standard antibiotics underscores the need for alternative treatments. Sida acuta, a plant with medicinal properties, was examined in this study for its potential antibacterial effects. This research assessed the phytochemical profile, performed Gas Chromatography-Mass Spectrometry (GC-MS) analysis, and evaluated the antibacterial efficacy of Sida acuta extracts against H. pylori. Leaves of S. acuta were extracted using hexane, ethanol, and water, and their phytochemical constituents were analyzed through established qualitative and quantitative methods. The antibacterial activity of the leaf extracts was tested against H. pylori via agar well diffusion and broth dilution using various extract concentrations. GC-MS analysis was conducted to identify the bioactive compounds in each extract. Phytochemical screening revealed the presence of alkaloids, saponins, glycosides, tannins, flavonoids, triterpenoids, steroids, and phenols in the extracts. Aqueous extracts exhibited the largest inhibition zones (20.23±0.16 mm) at 500 mg/mL, followed by ethanol (18.25±0.25 mm) and hexane (16.35±0.25 mm) extracts. Minimum inhibitory concentrations (MIC) were determined as 62.5 mg/mL for ethanol, 125 mg/mL for water, and 250 mg/mL for hexane extracts. Bactericidal effects were observed with the ethanol and aqueous extracts at 250 mg/mL and 500 mg/mL, respectively, while hexane showed no bactericidal activity at tested concentrations. GC-MS identified 27 compounds in the ethanol extract, 20 in the aqueous extract, and 25 in the hexane extract. These findings suggest that Sida acuta possesses antibacterial properties that may be beneficial in treating ulcer-related H. pylori infections.

Keywords: Antibacterial activity, GC-MS analysis, Phytochemical profiling, Sida acuta, Helicobacter pylori.

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a Gram-negative, helical, microaerophilic bacterium that colonizes the human gastrointestinal system (Mladenova, 2021), affecting nearly 50% of the global population and establishing itself as a significant pathogen. The World Health Organization has classified *H. pylori* as a Group I carcinogen due to its role in various gastrointestinal diseases, including non-cardiac gastric cancer, peptic ulcer disease, chronic and atrophic gastritis, and mucosa-associated lymphoid tissue (MALT) lymphoma (Malaoa, 2021).

Transmission of *H. pylori* occurs through multiple pathways, including direct person-to-person contact, fecal-oral and gastro-oral routes, as well as exposure to contaminated environments (Seth *et al.*, 2013). Risk factors for infection encompass socio-economic status, living conditions, age, poor hygiene, dietary habits, insufficient water quality, and limited health education (Hunt *et al.*, 2011). Conventional treatments for *H. pylori*-induced ulcers typically include proton pump inhibitors (PPIs), H_2 receptor antagonists, antacids, and antibiotics. However, these treatments often result in long-term side effects and carry a risk of recurrence, leading some patients to explore alternative therapeutic options (Dharamani & Palit, 2006).

Additionally, traditional therapies do not consistently fulfill all requirements for ulcer management (Dharmani & Palit, 2006).

With these limitations, herbal medicine is increasingly viewed as a promising alternative, particularly with plants like *Sida acuta*, known for their bioactive compounds. *Sida acuta*, commonly referred to as "stubborn weed" and belonging to the Malvaceae family, is a prominent plant in current research. Studies have identified a range of bioactive compounds in *Sida acuta*, including alkaloids, saponins, tannins, and flavonoids, which are believed to contribute to its medicinal properties (Femoe *et al.*, 2022). Traditionally, its extracts have been used to address various ailments, including malaria, diuretic conditions, and fever. Cytotoxicity and phytochemical analyses of *Sida acuta* hydroethanolic extracts indicate its potential for diverse biological applications (Femoe *et al.*, 2022). This plant, characterized by its erect, perennial shrub form with lanceolate leaves and yellow flowers, is easily identifiable (Usman &Abdulkarim, 2023).

MATERIALS AND METHODS

Source and Maintenance of Isolate

The *Helicobacter pylori* isolate used in this study was obtained from the University of Ilorin Teaching Hospital, Ilorin, Nigeria. The bacterial isolate was maintained on both Columbia agar and nutrient agar slants, stored at 4°C in a refrigerator, and sub-cultured weekly to ensure purity.

Collection of Plant samples

Sida acuta was collected from a private residence in the Agbabiaka area of Ilorin, Kwara State, Nigeria. The sampling location's coordinates are 8°27′52″N and 4°34′52″E. The plant was identified and authenticated at the Herbarium Unit, Department of Plant Biology, University of Ilorin, Kwara State, and registered with the reference number UILH/001/766/2024 for research purposes.

Extract Preparation

The leaves of *Sida acuta* were carefully rinsed with clean water to remove any impurities and subsequently air-dried for a period of two weeks. Once fully dried, the leaves were ground into a fine powder using a manual grinder. This powdered form was then stored in sterile plastic bags at ambient temperature for later extraction. For the extraction process, 100 grams of powdered *Sida acuta* material was added to conical flasks containing 500 mL each of water, ethanol, and n-hexane. The mixtures were stirred thoroughly to ensure uniformity and then subjected to continuous shaking for 48 hours on an auto shaker. Following this, the mixtures were filtered, with the aqueous filtrate concentrated using a water bath at 40°C to yield a crude fraction, while the ethanol and n-hexane filtrates were concentrated using a rotary evaporator to obtain their respective crude fractions (Waqas, 2021). The crude fractions were subsequently prepared by dissolving them in 3% Dimethyl Sulfoxide (DMSO) and performing serial dilutions from the stock solution (Ajijolakewu & Awarun, 2015).

Sensitivity Testing of Plant Extracts and Antibiotics Against *H.pylori*

Mueller-Hinton agar was prepared and poured into separate plates, which were then left to solidify. An inoculum suspension was created from an 18-hour culture of *H. pylori*, previously incubated at 37°C, and standardized to a turbidity equivalent to 0.5 on the McFarland scale. A sterile cotton swab was dipped into the standardized inoculum, and the agar plate surfaces were swabbed in a rotating manner to ensure even distribution. The plates were left to air-dry for about 20 minutes, after which a sterile 5 mm cork borer was used to create wells in each inoculated plate. Various dilutions of the plant extracts and antibiotics (ranging from 500 mg/mL to 2 mg/mL) were dispensed into the wells. Negative controls included water and 3% DMSO, while chloramphenicol served as the positive control. The plates were then incubated at 37°C for 24 hours, and the resulting zones of inhibition were measured in millimeters (mm) using a meter rule (Li *et al.*, 2024).

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was assessed using the tube dilution method to evaluate the ability of the test bacterium to grow in broth cultures containing varying concentrations of the extract (Molanaei *et al.*, 2020). A stock solution of *Sida acuta* extract at 1000 mg/mL was initially prepared, followed by dilution with an equal volume of sterile distilled water to produce a concentration of 500 mg/mL. Further serial dilutions were prepared by combining equal volumes of extract and sterile distilled water to yield concentrations of 250 mg/mL, 125 mg/mL, 62.5 mg/mL, 31.25 mg/mL, 16 mg/mL, 8 mg/mL, 4 mg/mL, and 2 mg/mL. Each dilution (2 mL) was then mixed with sterilized nutrient broth in sterile test tubes, and 50 µL of standardized bacterial inoculum was added to each tube. A positive control tube containing inoculum and nutrient broth but without extract and negative controls comprising tubes with only nutrient broth, or nutrient broth and extract but no inoculum, were also included.

All test tubes were incubated at 37°C for 24 hours. The MIC was identified as the lowest concentration of the extract showing no visible turbidity, indicating the inhibition of bacterial growth (Owuama, 2017).

Determination of Minimum Bactericidal Concentration (MBC)

All test tubes that showed no turbidity were further cultured on nutrient agar plates and incubated at 37°C for 24 hours. Following incubation, the plate with no visible colony growth and the lowest extract concentration was recorded as the minimum bactericidal concentration (MBC) (Tiwari *et al.*, 2018).

Phytochemical Screening

The pulverized *Sida acuta* samples were sieved to achieve a fine powder, and the extracts were subsequently filtered using Whatman filter paper.

The different extracts of *Sida acuta* were subjected to preliminary phytochemical screening by using standard procedures for the detection of Tannin, flavonoid, steroid, phenol, glycoside, saponin, and alkaloid (Adugna *et al.*, 2022; Devade *et al.*, 2022; Sharma *et al.*, 2022)

Quantitative Estimation

Determination of Total Phenolic Contents

Quantitative estimation of phytochemicals was conducted to determine total phenols, tannins, flavonoids, saponin, alkaloids, glycoside, steroid, and terpenoid contents (Chowdhury, 2021; Hussain *et al.*, 2013; Shraim *et al.*, 2021).

Gas Chromatography/Mass Spectrometry (GC-MS) Analysis

GC-MS analysis was performed using an Agilent hyphenated gas chromatography system connected to a mass spectrometer with a triple-axis detector and equipped with an auto-injector. The system had two capillary columns coated with phenyl methyl silox (fused silica, 25 m × 0.25 mm, 0.20 µm film thickness) and a flame ionization detector (FID). A volume of 1.00 µLwas injected with a split ratio of 1:50. The oven temperature was programmed to rise from 35°C to 250°C at a rate of 5°C/min, with hydrogen as the carrier gas. The injection and detector temperatures were set at 300°C and 250°C, respectively. Mass spectra were compared with standard spectra from the NIST library. The percentage composition of each compound was determined based on the GC peak areas (Zakariyah *et al.*, 2017).

RESULTS

Table 1 revealed the antibacterial activities of various leaf extracts of *Sida acuta* (Ethanolic, Aqueous, and Hexane) against *Helicobacter pylori*, as measured by the zone of inhibition in millimeters (mm).

At the highest concentration of 500 mg/mL, the ethanolic extract showed a zone of inhibition of 18.25 ± 0.25 mm, the aqueous extract exhibited 20.23 ± 0.16 mm, and the hexane extract showed 16.35 ± 0.25 mm, while Chloramphenicol displayed 25.03 ± 0.03 mm. As the concentration decreased to 250 mg/mL, the inhibition zones decreased to 16.10 ± 0.10 mm, 16 ± 0.00 mm, and 15 ± 0.00 mm for the ethanolic, aqueous, and hexane extracts, respectively, with Chloramphenicol showing 18 ± 0.00 mm. At 125 mg/mL, the ethanolic, aqueous, and hexane extracts exhibited inhibition zones of 15 ± 0.00 mm, 15 ± 0.00 mm, and 12 ± 0.00 mm, respectively, while Chloramphenicol showed 16.04 ± 0.04 mm. At 62.5 mg/mL, the ethanolic extract exhibited a zone of inhibition of 13 ± 0.00 mm, while no inhibition was observed for the aqueous and hexane extracts. Chloramphenicol showed 15.05 ± 0.05 mm of inhibition. At 31.25 mg/mL, only the ethanolic extract displayed inhibition (10.15 ± 0.15 mm), with no inhibition from the aqueous and hexane extracts. Chloramphenicol showed 12 ± 0.00 mm. At the lowest concentration of 15.63 mg/mL, only Chloramphenicol demonstrated inhibition (10 ± 0.00 mm), with all extracts showing no inhibition.

The ethanolic extract exhibited the lowest MIC of 62.5 mg/mL and an MBC of 250 mg/mL, indicating the highest effectiveness against *H. pylori*. The aqueous extract showed a moderate effect with an MIC of 125 mg/mL and an MBC of 500 mg/mL, while hexane extract had an MIC of 250 mg/mL with no observed bactericidal effect, making it the least effective.

The analysis of qualitative phytochemical screening revealed that the crude extract contains high levels of alkaloids, tannins, phenolics, and glycosides, with moderate amounts of triterpenoids, steroids, and saponins. Flavonoids were found in low amounts while reducing sugars and proteins were completely absent in the crude extract (Table 3).

The quantitative phytochemical analysis of the crude extracts revealed the presence of higher levels of phenols (138.6 mg/100g ± 0.02) and flavonoids (3.7 mg/100g ± 0.00), but lower amounts of alkaloids (55.7 mg/100g ± 0.00), steroids (17.8 mg/100g ± 0.04), triterpenoids (27.3 mg/100g ± 0.01), and glycosides (62.1 mg/100g ± 0.02). There were no measurable

amounts of reducing sugars and proteins in the GC-MS analyses of *Sida acuta* extracts in ethanolic, aqueous, and hexane solvents showed the presence of various bioactive compounds that could contribute towards the medicinal properties of the plant (Table 5, 6, 7). The first and predominant compound identified in the aqueous extract with less retention time (7.557 mins) was Butanoicacid, whereas Scopolamine was the last compound identified which took the longest retention time (26.154 mins) for identification. Formicacid,1-methylethyl ester (8.011 mins), and Hydroperoxide, 1-ethylbutyl (9.048 mins) were the first compounds to be identified in the ethanolic and hexane extracts, respectively.

Table 1: Antibacterial activities of the leaf extracts of *Sida acuta* on *Helicobacter pylori*

Concentration	Zone Diameter of inhibition of extracts/ Antibiotics (mm)			
(mg/ml)	Ethanolic	Aqueous	Hexane	Chloramphenicol
500	18.25 ± 0.25	20.23 ± 0.16	16.35 ± 0.25	25.03 ± 0.03
250	16.10 ± 0.10	16 ± 0.00	15 ± 0.00	18 ± 0.00
125	15 ± 0.00	15 ± 0.00	12 ± 0.00	16.04 ± 0.04
62.5	13 ± 0.00	0.00	0.00	15.05 ± 0.05
31.25	10.15 ± 0.15	0.00	0.00	12 ± 0.00
15.63	0.00	0.00	0.00	10 ± 0.00

Key: 0.00 in the table depicts no zone of inhibition

Table 2: Minimum Inhibitory and Bactericidal concentrations of *Sida acuta* against *H.pylori*

Organism	Observation *Sida acuta* leaves extracts					
	Ethanol		Aqueous		Hexane	
H.pylori	MIC	MBC	MIC	MBC	MIC	MBC
	62.5	250	125	500	250	NIL

Values are in mg/ml

Table 3: Qualitative Phytochemical analysis of crude extracts of *Sida acuta*.

Phytochemical	Observation *Sida acuta*
Phenol	+++
Saponin	++
Tannin	+++
Alkaloids	+++
Flavonoids	+
Steroids	+++
Triterpenoids	++
Glycoside	+++
Reducing sugar	---
Proteins	---

Key: +++ indicates the compound was present in significant amounts, ++ signifies it was found in moderate quantities, + represents trace amounts, and --- denotes the compound was absent.

Table 4: Quantitative Phytochemical Analysis of Crude Extracts *Sida acuta*

Phytochemical	Observation *Sida acuta* Value (mg/mL)
Phenol	138.6 ± 0.02
Saponin	12.3 ± 0.03
Tannin	119.7 ± 0.01
Alkaloids	55.7 ± 0.00
Flavonoids	3.7 ± 0.00
Steroids	17.8 ± 0.04
Triterpenoids	27.3 ± 0.01
Glycoside	62.1 ± 0.02
Reducing sugar	0.00 ± 0.00
Proteins	0.00 ± 0.00

Table 5: GCMS analysis of *Sida acuta* ethanolic extract showing diverse chemical elements

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
1	8.011	1200178	1.58	443012	1.88	2.71	Formicacid,1-methylethyl ester
2	12.455	2114486	2.78	303581	1.29	6.97	1-Dodecene
3	12.597	1851827	2.44	622284	2.64	2.98	1-Dodecene
4	12.675	569232	0.75	222793	0.95	2.55	Tridecane
5	13.840	1050668	1.38	113521	0.48	9.26	Decane, 2-methyl-
6	13.996	1845241	2.43	678416	2.88	2.72	Hexadecane
7	15.055	3081827	4.06	355814	1.51	8.66	7-Heptadecene,1-chloro-
8	15.191	4926418	6.49	1542716	6.56	3.19	1-Dodecene
9	15.240	2239003	2.95	993694	4.22	2.25	Tridecane
10	16.439	783832	1.03	361995	1.54	2.17	Tetradecane
11	16.842	858767	1.13	295495	1.26	2.91	Phenol,2,6-bis(1,1-dimethylethyl)-
12	17.516	3771970	4.97	1337599	5.68	2.82	Cetene
13	18.604	840174	1.11	297973	1.27	2.82	Hexadecane
14	19.646	2410369	3.17	832120	3.54	2.90	3-Eicosene,(E)-
15	20.349	1910451	2.52	755946	3.21	2.53	2-Pentadecanone,6,10,14-trimethyl-
16	21.168	7100782	9.35	2477826	10.53	2.87	Hexadecanoicacid,methyl ester
17	21.620	1213490	1.60	113890	0.48	10.65	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien
18	21.795	3514833	4.63	884482	3.76	3.97	1,2-Benzenedicarboxylicacid,butyl8-methyln
19	21.868	8551994	11.26	2800737	11.90	3.05	Hexadecanoicacid, ethylester
20	21.982	9731662	12.82	3032966	12.89	3.21	Dibutyl phthalate
21	23.235	1285946	1.69	416134	1.77	3.09	14,17-Octadecadienoic acid,methylester
22	23.386	4899949	6.45	1362615	5.79	3.60	Phytol
23	23.470	2121891	2.79	700123	2.97	3.03	Methylstearate
24	24.029	4644295	6.12	1392944	5.92	3.33	(E)-9-Octadecenoicacidethylester
25	24.277	2776159	3.66	939171	3.99	2.96	Octadecanoicacid,ethylester
26	25.621	223851	0.29	102325	0.43	2.19	1-Hexacosene
27	25.830	415651	0.55	154216	0.66	2.70	Cyclo-(glycyl-l-leucyl)
		75934946	100.00	23534388	100.00		

Table 6: GCMS analysis of *Sida acuta* aqueous extract showing diverse chemical elements

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
1	7.557	3287455	8.19	1046162	8.84	3.14	Butanoicacid
2	10.038	2256150	5.62	603319	5.10	3.74	Phenol
3	11.287	1104518	2.75	234177	1.98	4.72	2-Pentanone,1-phenyl-
4	12.670	2053863	5.12	674092	5.70	3.05	1-Dodecene
5	15.220	3659099	9.12	1404609	11.87	2.61	1-Tridecene
6	17.524	2785889	6.94	1119716	9.46	2.49	Cetene
7	19.648	1092098	2.72	384577	3.25	2.84	1-Heptadecene
8	20.125	531797	1.33	122701	1.04	4.33	Carbonicacid,propylundec-10-enylester
9	20.340	7008447	17.47	1975478	16.69	3.55	2-Pentadecanone,6,10,14-trimethyl-
10	20.475	719865	1.79	217895	1.84	3.30	Octadecanoicacid,ethylester
11	20.639	645055	1.61	132265	1.12	4.88	1-Heptadec-1-ynyl-cyclopentanol
12	20.876	840477	2.09	188894	1.60	4.45	1,2-Benzenedicarboxylicacid,bis(2-methylpro
13	21.163	1551593	3.87	522077	4.41	2.97	Hexadecanoicacid,methylester
14	21.863	4356667	10.86	1338585	11.31	3.25	Hexadecanoicacid,ethylester
15	23.375	2276772	5.67	616258	5.21	3.69	Phytol
16	24.006	1647846	4.11	402389	3.40	4.10	9,12-Octadecadienoicacid,ethylester
17	24.134	1342767	3.35	315543	2.67	4.26	9,12,15-Octadecatrienoicacid,ethylester,(Z,
18	24.261	1608712	4.01	274059	2.32	5.87	Octadecanoicacid,ethylester
19	24.725	709127	1.77	123536	1.04	5.74	9,12,15-Octadecatrienoicacid,ethylester,(Z,
20	26.164	650311	1.62	139973	1.18	4.65	Scopolamine
		40128508	100.00	11836305	100.00		

Table 7: GCMS analysis of *Sida acuta* Hexane extract showing diverse chemical elements

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
1	9.048	21420056	10.14	3540960	6.15	6.05	Hydroperoxide, 1-ethylbutyl
2	9.182	32790660	15.52	4284023	7.45	7.65	Hydroperoxide,1-methylpentyl
3	13.516	5302541	2.51	643657	1.12	8.24	n-Tridecylcyclohexane
4	13.614	2510694	1.19	984678	1.71	2.55	Octane,2,3,7-trimethyl-
5	13.670	1975126	0.93	787558	1.37	2.51	Dodecane,4-methyl-
6	14.018	3575447	1.69	1609871	2.80	2.22	Tridecane
7	14.932	2829724	1.34	952631	1.66	2.97	Nonane,3-methyl-5-propyl-
8	15.258	6861024	3.25	2542383	4.42	2.70	Hexadecane
9	16.451	3197608	1.51	1447286	2.52	2.21	Pentadecane
10	16.603	11515218	5.45	4405900	7.66	2.61	2,4a-Methanonaphthalen-7(4aH)-one,1,2,3,4,
11	16.846	3834991	1.82	1080696	1.88	3.55	Phenol,2,4-bis(1,1-dimethylethyl)-
12	17.561	3985387	1.89	1500251	2.61	2.66	Pentadecane
13	18.616	7551761	3.57	2212457	3.85	3.41	Heptadecane
14	19.682	2787859	1.32	635745	1.11	4.39	Octadecane
15	20.150	1985068	0.94	617427	1.07	3.22	2,3,5,6-Detetrahydrocyclohexanone,2,6-di-t-b
16	20.700	3001658	1.42	410493	0.71	7.31	1,3-Dihydrobenzimidazol-2-one,N,N'-blis(ter
17	20.829	2446461	1.16	894901	1.56	2.73	Nonane,5-methyl-5-propyl-
18	21.871	22868405	10.82	7446577	12.94	3.07	Hexadecanoicacid, ethylester
19	21.986	26425046	12.51	8495306	14.77	3.11	Dibutylphthalate
20	22.860	4579371	2.17	1648705	2.87	2.78	1,3-Dihydrobenzimidazol-2-one,N,N'-blis(ter
21	23.166	3459277	1.64	868888	1.51	3.98	Eicosane
22	23.386	2519651	1.19	867029	1.51	2.91	Phytol
23	24.017	8930971	4.23	2460522	4.28	3.63	9,12-Octadecadienoicacid(Z,Z)-
24	24.141	4935024	2.34	1323141	2.30	3.73	9,12,15-Octadecatrienoicacid,ethylester,(Z,
25	24.279	20004506	9.47	5869930	10.20	3.41	Octadecanoicacid,ethylester
		211293534	100.00	57531015	100.00		

DISCUSSION

Medicinal plants have been utilized for centuries due to their therapeutic properties, yielding compounds with recognized antimicrobial activity. These compounds can effectively inhibit or eliminate pathogens while minimizing harm to host cells, making them promising candidates for developing new antimicrobial agents (Priya *et al.*, 2021). This study compared the in vitro antimicrobial efficacy of *Sida acuta* leaf extracts against *H. pylori*.

Table 1 presents the antimicrobial activities of *Sida acuta* extracts at various concentrations. The ethanolic extract exhibited the highest efficacy, indicating strong inhibitory and bactericidal properties; the aqueous demonstrated moderate activity, while n-Hexane displayed minimal efficacy, with no observed bactericidal activity. The antimicrobial activity of the ethanolic extract is consistent with the findings of Ekwealor *et al.* (2020), who reported similar inhibition zones against *H. pylori*, suggesting that ethanol is an effective solvent for extracting antimicrobial compounds. The enhanced efficacy of both the aqueous and ethanolic extracts of *Sida acuta* leaves can be attributed to the polar nature of these solvents, which are capable of efficiently extracting a wide range of polar compounds. This process leads to higher concentrations of bioactive antimicrobial agents in the extracts. Furthermore, both aqueous and ethanolic extracts promote the penetration of antimicrobial compounds into microbial cells, facilitating interactions with cell membranes and ultimately resulting in cell lysis and death (Bassey *et al.*, 2021).

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values for *Sida acuta* extracts are presented in Table 2. The ethanolic extract exhibited the highest efficacy, with a MIC of 62.5 mg/ml and an MBC of 250 mg/ml, indicating strong inhibitory and bactericidal properties. The aqueous extract demonstrated moderate activity, with a MIC of 125 mg/ml and an MBC of 500 mg/ml.

The n-Hexane extract displayed minimal efficacy, with a MIC of 250 mg/ml and no observed bactericidal activity. The superior performance of the ethanolic extract suggests its potential as a potent antimicrobial agent. This high efficacy can be attributed to the ethanolic extract's rich content of bioactive compounds. Ethanolic extracts are known to contain a diverse range of compounds that contribute to their broad-spectrum antimicrobial effects, enhancing their ability to combat various microorganisms (Bozinou, 2023). Further research into the chemical composition and mechanisms of action of the ethanolic extract is needed to fully understand its effectiveness.

The qualitative and quantitative phytochemical analyses presented in Tables 3 and 4 offer valuable insights into the compounds responsible for the observed antimicrobial activity. *Sida acuta* extracts contain high concentrations of phenolics and alkaloids, which are recognized for their antimicrobial properties, aligning with the findings of Al-Snafi (2017a). Phenolic compounds can alter cell permeability, leading to leakage of cellular macromolecules, and may disrupt membrane proteins, causing both structural and functional changes. Additionally, higher concentrations and the combination of various phenolic compounds enhance their antimicrobial activity (Khoury *et al.*, 2017). Alkaloids have been shown to intercalate with DNA, further contributing to their antimicrobial effects (Al-Snafi, 2017b). Flavonoids and terpenes, known for their antioxidant properties, also play a role in the plant's antimicrobial activity (Hill *et al.*, 2011). The significant levels of phenolics and alkaloids found in *Sida acuta* extracts, particularly in the ethanolic extract, support their antimicrobial potential against *H. pylori*. These findings align with those of Shittu and Alagbe (2020), underscoring the diverse and multifaceted nature of plant-derived compounds in combating *H. pylori*-mediated ulcers (Rostami & Haddadi, 2022).

The GC-MS analysis of *Sida acuta* ethanolic extract, as shown in Table 5, highlights the chemical composition, retention times, and relative abundances of the identified compounds. A total of 27 distinct peaks were detected, each corresponding to a different compound. The most abundant compounds, based on their percentage Area, include Dibutyl phthalate (12.82%), Hexadecanoic acid, ethyl ester (11.26%), and Hexadecanoic acid, methyl ester (9.35%). Other notable components include Phytol (6.45%) and (E)-9-Octadecenoic acid ethyl ester (6.12%). The retention times of these compounds vary, reflecting their unique chemical properties. For example, Formic acid, 1-methylethyl ester elutes early at 8.011 minutes, while Cyclo-(glycyl-l-leucyl) appears later at 25.830 minutes. The area and height of each peak provide quantitative insights into the concentration of each compound, and the Area-to-Height (A/H) ratio offers additional information about peak shape. For instance, 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien exhibits a high A/H ratio of 10.65, indicating a broader peak, whereas compounds such as 1-Dodecene display lower A/H ratios, suggesting sharper peaks.

The GC-MS analysis of *Sida acuta* aqueous extract, shown in Table 6, reveals 20 active compounds. Among these, 2-Pentadecanone, 6,10,14-trimethyl- stands out as the most abundant with an area percentage of 17.47%, followed by Hexadecanoic acid, ethyl ester (10.86%) and 1-Tridecene (9.12%). Minor constituents, such as Scopolamine, present in lower amounts (1.62%), were also detected. Noteworthy peaks, such as Butanoic acid and Cetene, exhibit A/H ratios of 3.14 and 2.49, respectively, which may indicate sharper peaks.

The GC-MS analysis of *Sida acuta* n-hexane extract, shown in Table 7, identifies 25 compound peaks. Among these, Hexadecanoic acid ethyl ester (Peak 18) at 10.82%, Dibutyl phthalate (Peak 19) at 12.51%, and Octadecanoic acid ethyl ester (Peak 25) at 9.47% are the most notable. These compounds, ranging from esters and fatty acids to hydrocarbons like alkyl and cycloalkanes, vary in structure and functionality. Each peak is characterized not only by its area and height, reflecting the concentration and purity of the compound, but also by its relative contribution to the overall composition of the extract.

CONCLUSION

This study highlights the potential of *Sida acuta* leaf extracts as effective antimicrobial agents against *Helicobacter pylori*. The ethanolic extracts demonstrated significant bactericidal activity, indicating the presence of bioactive phytochemicals capable of inhibiting and lysing *H. pylori*. Phytochemical profiling revealed key compounds, including phenols, alkaloids, tannins, and flavonoids, known for their antimicrobial properties. GC-MS analysis identified specific phytoconstituents that may contribute to these effects. These findings confirm the antimicrobial potential of *Sida acuta* and its significance in addressing *H. pylori*-associated diseases, offering a promising natural therapeutic alternative in combating antibiotic resistance.

This study emphasizes the importance of further research to determine the mechanisms of action of these bioactive compounds, optimize extraction methods, and evaluate the clinical efficacy of *Sida acuta* formulations. By building on traditional medicinal knowledge, this research supports the use of medicinal plants in the development of effective therapeutic agents for bacterial infections.

RECOMMENDATION

Further studies should focus on understanding the mechanisms of *Sida acuta* extracts against *H. pylori*. Clinical trials are needed to confirm safety and efficacy in humans. Optimizing extraction methods and creating standardized formulations will ensure consistent quality. Combining *Sida acuta* extracts with antibiotics could enhance treatment effectiveness. Promoting sustainable cultivation practices will support a reliable supply for future use.

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