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IN VITRO EVALUATION OF THE ANTIBACTERIAL EFFICACY OF *DATURA STRAMONIUM* LEAF AGAINST *HELICOBACTER PYLORI*

***[1]Ajadi, A.E., †[2]Ajijolakewu, A. K., [3]Ayoola, S. A., [2]Omotunde, M. Y. and [2]Aguda, B. A.**

[1]Department of Microbiology; Federal University of Health Sciences, Ila-Orangun, Osun State, Nigeria
[2]Department of Microbiology, Faculty of Life Sciences, University of Ilorin, Kwara State, Nigeria.
[3]Bioresources Development Centre, National Biotechnology Development Agency, Ogbomosho, Oyo State, Nigeria.

*Corresponding authors' email: ajadi.ayobami.aa@gmail.com Phone: +2348182222994
ORCID iD:* https://orcid.org/0009-0005-3356-9505 † https://orcid.org/0000-0002-0007-9623

ABSTRACT

The rise in antimicrobial resistance has spurred the search for plant-based alternatives due to their therapeutic properties. This study evaluates the *in vitro* efficacies of *Datura stramonium l*eaves extracts against *Helicobacter pylori*. Leaves were extracted using selected polar and non-polar solvents, and antibacterial activity were assessed through the agar well diffusion method at varying concentrations with *H.pylori* as test bacteria. Phytochemical analysis was conducted using standard methods. Results showed that at 500 mg/ml, ethanolic extracts of *Datura stramonium* showed the highest zone of inhibition (22.02 ± 0.02 mm), followed by n-Hexane (17.03 ± 0.03 mm) and aqueous extracts (15.03 ± 0.03 mm). Ethanolic and n-Hexane of *Datura stramonium* extracts had high MIC values (125 mg/ml), while the aqueous extract had 250 mg/ml. Ethanolic extract and n-Hexane extracts of *Datura stramonium* also had bactericidal activity at 500 mg/ml, unlike the aqueous extracts. Phytochemical analysis revealed the presence glycosides (74.4± 0.04) mg/ml, phenols (131.8±0.03) mg/ml, alkaloids (100.5±0.00) mg/ml flavonoids (2.6±0.02) mg/ml, tannins (124.2±0.02) mg/ml, saponins (14.1±0.02) mg/ml, triterpenoids (37.9±0.00) mg/ml, and steroids (31.9±0.01) mg/ml in the extracts. The results showed that *Datura stramonium* leaf ethanolic extracts had strong antibacterial activity against *H. pylori*, suggesting that they could be used as an alternative to conventional therapies for *H. pylori* infections.

Keywords: Antimicrobial resistance, Antibacterial activity, *Datura stramonium*, *Helicobacter pylori*

INTRODUCTION

The discovery of *Helicobacter pylori* fundamentally challenged the long-standing belief that the stomach's acidic environment was sterile. In 1982, Warren and Marshall successfully isolated the bacterium responsible for chronic gastritis, despite earlier evidence of spiral-shaped, Gram-negative, microaerophilic organisms in the stomach (Malfertheiner *et al*., 2023). Today, *H. pylori* is recognized as one of the most prevalent infections, affecting nearly half of the global population by colonizing the gastrointestinal mucosa (Ahmed & Mohammed, 2018). Its ability to establish chronic, often lifelong, infections in the stomach lining underscores its reputation as a formidable pathogen. The bacterium's remarkable adaptation to the stomach's harsh acidity triggers a range of immune responses and pathological consequences (Zhang *et al*., 2021). While initially associated with peptic ulcers, *H. pylori* has since been linked to a variety of extra-gastric conditions, including neurological, ocular, hematologic, cardiovascular, and dermatological disorders, all of which contribute to significant global healthcare and economic burdens. Furthermore, it is implicated in more serious conditions such as gastritis, duodenal ulcers, gastric cancer, and mucosa-associated lymphoid tissue (MALT) lymphoma (Zha *et al.*, 2022). The severity of *H. pylori*-related symptoms is influenced by a combination of factors, including the host's immune response, the virulence of bacterial strains, and environmental influences such as co-infections, diet, stress, and hygiene practices (Hsu *et al.*, 2021). Additional risk factors, including racial background, socioeconomic status, rural residency, poor sanitation, inadequate food safety, water scarcity, and lack of awareness, further complicate the transmission dynamics, which occur primarily through person-to-person contact or via fecal-oral and gastro-oral routes (Qiu *et al.*, 2021).

Conventional treatment options for *H. pylori*-related ulcers, such as proton pump inhibitors (PPIs), H2 receptor antagonists, antacids, and antibiotics have shown limitations, including the development of drug tolerance, long-term side effects, and high recurrence rates (Hamidi *et al.*, 2020). These challenges have spurred interest in alternative therapies, particularly herbal remedies that offer natural ingredients with fewer side effects. Herbal medicine is increasingly viewed as a viable alternative to pharmaceutical drugs due to its availability, affordability, efficacy, and safety profile (Panezai *et al.*, 2021). Scientific research has demonstrated that numerous tropical plants possess significant anti-ulcer properties, with many dietary and medicinal plants showing gastroprotective effects. The therapeutic potential of plant-based treatments has gained considerable attention, especially in the context of *H. pylori* infections, a major cause of intestinal ulcers. Though specific studies on *Datura stramonium* commonly known as Jimsonweed are limited regarding its direct effects on *H. pylori*, research on related plant extracts provides promising insights (Baylie *et al.*, 2023). *Datura stramonium*, a member of the Solanaceae family, has been extensively studied for its pharmacological properties, revealing significant antioxidant, antihyperglycemic, and antihyperlipidemic effects, suggesting its potential in treating metabolic disorders (Baylie *et al.*, 2023). Moreover, its demonstrated antibacterial activity against pathogens such as *Shigella* species and *Salmonella typhi* suggests potential relevance in treating bacterial infections, including those affecting the gastrointestinal system (Imarenezor *et al.*, 2022). The plant's therapeutic properties are attributed to its rich phytochemical composition, which includes alkaloids, flavonoids, withanolides, and sesquiterpenes, highlighting its potential role in medicinal applications (Dike-Ndudim *et al*., 2021).

Ulcers remain a prevalent and serious health challenge, particularly among vulnerable populations such as the young, weak, and elderly (Onadeko & Akinola, 2021). The growing resistance of *Helicobacter pylori* to conventional antibiotics has exacerbated treatment failures, insufficient eradication, and recurrent infections. This alarming trend necessitates innovative solutions, prompting the exploration of alternative therapies derived from native plants with inherent antibacterial properties. In this study, *Datura stramonium* leaf was evaluated *in vitro* for its antibacterial efficacy against *H. pylori*.

MATERIALS AND METHODS

Bacterium Origin and Maintenance

Helicobacter pylori was bacterium of interest utilized for this investigation. The bacterium isolate was acquired in a sterile bottle on the 6th of march, 2024 at University of Ilorin teaching hospital in Ilorin, Kwara State, Nigeria. In order to preserve purity, the bacterial isolate was kept on nutrient agar slant, refrigerated at 4˚C, and Sub-cultured once a week.

Plant Sample collection

Datura stramonium leaves were obtained from a private residence in Agbabiaka Area, Ilorin, Kwara State, Nigeria. Latitude 8°27′52″N and longitude 4°34′52″E are the coordinates of the sample location. The plant was recorded for research purposes under the authorization numbers UILH/002/1256/2024, respectively, after being recognized and certified at the Herbarium unit of the Department of Plant Biology, University of Ilorin, Kwara State, Nigeria.

Preparation of the extract

After completely cleaning the leaves of *Datura stramonium* with clean water to get rid of any impurities, they were allowed to air dry for two weeks. Afterwards, Qasa manual machine was used to grind them into a fine powder. For later usage, the powdered leaves were kept in sterile plastic container at room temperature. Five hundred millilitres of ethanol, n-hexane, and water were added to conical flasks containing 100 grammes of the powdered plant material was used for the extraction process. Thereafter, mixtures were shaken manually to ensure homogeneity, then placed on an autoshaker for continuous shaking at 180 RPM for 48 hours. Complete homogeneous samples were then filtered using Whatman No. 1 filter paper into conical flasks. The crude extract was obtained by evaporating the aqueous filtrate in a water bath at 40°C. To get the corresponding crude extracts, filtrates from ethanolic and N-hexane solvent were concentrated using a rotary evaporator (Waqas, 2021). The next step involves dissolving the crude extracts in 3% Dimethyl Sulfoxide (DMSO4) and serially dilution of the stock solution (Ajijolakewu & Awarun, 2015).

Sensitivity Test

Mueller Hinton agar was prepared, sterilized in an autoclave at 121^0C for 15mins, thereafter, maintained at 45^0C in a water bath and poured aseptically to various sterile petri dish and allowed to set. McFarland Standard of 0.5 turbidity was used to make and standardize the inoculum suspension from an 18-hour-old culture of the test *Helicobacter pylori* that was incubated in a sterile test tube at 37 ˚C. Thereafter, sterile cotton swab stick was dipped into the standardized inoculum and used to streak the surfaces of the agar plates for inoculation of the *Helicobacter pylori* then rotated periodically to ensure effective distribution. After 20 minutes of drying time, each of the inoculated agar plates had a hole drilled into it using a 5 mm diameter sterile cork borer. Antibiotics and plant extracts were diluted and poured into the wells at concentrations ranging from 2 mg/ml to 500 mg/ml (Genre *et al.,* 2015). Standard antibiotics (chloramphenicol) were utilized as the positive control, and water and 3% DMSO were utilized as the negative controls. For twenty-four hours, the plates were incubated at 37°C. The metre rule was then used to measure the zones of inhibitions in millimeters (mm) (Li et al., 2024).

Determination of Minimum Inhibitory concentration

The capacity of the test bacteria to grow in broth cultures with varying concentrations of the extract was used to calculate the Minimum Inhibitory Concentration (MIC) using the tube dilution method (Zulkipli *et al.,* 2022). The extract was serially diluted to progressively lower concentrations: 500 mg/ml, 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, respectively. Test tubes were sterilized and equal volumes (2 ml) of each diluted concentrations of extract and nutritional broth were poured into each tube. To every test tube, 50 µl of a standardized inoculum was introduced. The positive control was a test tube with no extract but an inoculum and nutrient broth, while the negative control was a test tube with no inoculum but nutrient broth and extract. As a negative control, another tube containing nutrient broth alone, both extract and inoculum was also utilized. Afterwards, the mixtures were incubated at 37°C for 24 hours. The minimal inhibitory concentration was determined by observing the lowest concentration of extract that did not exhibit any turbidity, indicating that bacterial growth was inhibited (Zulkipli *et al.,* 2022).

Determination of Minimum Bactericidal concentration

All the test tubes that showed no turbidity for Minimum Inhibitory Concentration (MIC) were plated onto nutrient agar and incubated for 24 hours at 37°C. After incubation, the plates with no single colony growth was noted with its corresponding concentration and taken as the Minimum Bactericidal Concentration (Owuama, 2017).

Preparation of Reagents for Phytochemical Screening

The reagent used for the phytochemical screening was prepared using standard method for the preparation of reagent of Dragendorff's Reagent, Mayer's reagent and Wagner's reagent (Sabdoningrum *et al.,* 2021, Godlewska *et al.,* 2022, Thi *et al.,* 2021).

Phytochemical Characterization

In order to obtain a fine powder, pulverized samples of *Datura stramonium* solution was sieved. Whatman filter No. 1 paper was used to filter the extracts.

Test for Tannins

Two millilitres of each extract were mixed with a few drops of 1% lead acetate, and the emergence of a dark green colour which denotes the presence of tannins was recorded (Khan *et al.,* 2023).

Test for Flavonoids

A portion of the sample extract was mixed with five millilitres of diluted ammonia, and then concentrated sulphuric acid was added. The presence of flavonoids is indicated by a yellow colouration (Shabira *et al.,* 2022)

Test for phenols

In five millilitres of distilled water, the extract was dissolved. A small amount of neutral 5% ferric chloride solution was

added to this. The phenolic component was characterized by a dark green colour (Ismail & Ali, **2022**).

Test for Glycosides

In a test tube containing 5 ml of extract from each plant sample, 1 ml of concentrated H_2S0_4 is formed and dissolved in 2 ml of glacial CH_3CO_2H that contains 1 drop of $Fecl_3$. One millilitre of concentrated H_2S0_4 is gently added to the mixture above, ensuring that the concentrated H_2S0_4 is underneath the mixture. If the sample contains glycoside, a brown ring colouration will appear up (Cui, 2024).

Test for saponin

A test tube is filled with two millilitres (2 ml) of extract, 10 ml of distilled water, and shaken for two minutes. a foamy appearance indicates the presence of saponin (Owusu *et al.,* 2021).

Test for Alkaloids

Two millilitres of extract and five millilitres of 2% HCL was dispense in the test tube and thoroughly mixed, warmed for a few minutes, filtrated and Wagner reagent was added. The presence of alkaloid causes the precipitate to form orange-red (Ren *et al.,* 2022).

Determination of selected plant contents of the *Datura stramonium* extract

Determination of Total Phenolic Contents

The Folin-Ciocalteu reagent was used to calculate the total phenolic content of the extracts. The total phenolics were reported as mg/g gallic acid equivalents (GAE), with gallic acid serving as the benchmark. A millilitre of methanol was used to make a standard solution containing 0.01, 0.02, 0.03, 0.04, and 0.05 mg/ml of gallic acid. Plant extract was also produced at concentrations of 0.1 and 1 mg/ml in methanol. 0.5 ml of each sample was added to test tubes along with 2.5 ml of a diluted Folin-Ciocalteu reagent (10 fold) and 2 ml of 7.5% sodium carbonate. After the test tubes were wrapped in parafilm and left to stand at room temperature for thirty minutes, the absorbance was measured spectrophotometrically at 760 nm (Rahman *et al.,* 2022).

Determination of Tannins contents

The Van-Burden and Robinson. (1981) method was used to determine the sample's tannin content. Five hundred mg of the sample was taken and placed in a 500 ml flask. Fifty ml of distilled water was added, and the mixture was shaken for one hour. The filtrate was pipetted out into a test tube and combined with 2 ml (10 times diluted) of 0.1 M $Fecl_3$ in 0.1N HCL and 0.008M potassium ferrocyanide after being filtered into a 50 ml volumetric flask. In less than ten minutes, a spectrophotometer at 605 nm was used to test the sample's absorbance (Zhang *et al*., 2017).

Determination of Total Flavonoids

The aluminium chloride technique was employed to determine the flavonoid content. Quercetin served as the standard in this approach, and the flavonoid levels were quantified in terms of quercetin equivalent. A 10-milliliter volumetric flask holding four millilitres of distilled water was filled with one millilitre of standard or extract solution (20, 40, 60, 80, or 100 mg/l). Zero point three millilitres of 5% $NaNO_2$ was added to the flask. Zero point three millilitres of 10% $AlCl_3$ was added to the mixture after 5 minutes. Two millilitres of 1M NaOH was added to make up the volume to 10 millilitres with distilled water at the 6-minute mark. The absorbance was measured with a UV-visible spectrophotometer at 510 nm (Gurung, 2019).

Determination of Saponin Content

After adding 0.5 g of the sample to 20 ml of $1NHCl_2$, the mixture was allowed to boil for four hours. It was filtered after cooling, and 50 millilitres of petroleum ether was added to the filtrate for the ether layer before being evaporated to dryness. To the residual, 5 millilitres of acetone ethanol were added. Each of the three test tubes held 0.4 ml of the substance. After adding 6 ml of ferrous sulphate reagent, 2 ml of concentrated H_2S0_4 was added. After ten minutes, it was well combined, and the absorbance at 490 nm was measured. The calibration plot curve was created using Standard Saponin (Hamid *et al.,* 2021).

Determination of Total Alkaloid Content

The total alkaloid content of the samples was measured using 1, 10-phenanthroline technique, described by (Kamel *et al.,* 2015). Ten ml of 80% ethanol-was used to extract 100 mg of the sample powder. This was centrifuged at 5,000 rpm for 10 min. The recovered supernatant was utilized to estimate total alkaloids further. One ml of plant extract, 1 ml of 0.025 M $FeCl_3$ in 0.5 M HCL, and 1 ml of 0.05 M 1, 10-phenanthroline in ethanol were all present in the reaction mixture. The mixture was incubated in a hot water bath at a constant temperature of 70 ± 2°C for 30 minutes. At 510 nm reagent blank, the absorbance of the red complex was measured. Using the quinine standard curve (0.1 mg/ml, or 10 mg dissolved in 10 ml ethanol and diluted to 100 ml with distilled water), the alkaloid levels were determined. The results were given as milligrammes per gramme of dry weight.

Determination of Glycoside

The glycoside was determined using (Mbah *et al.,* 2019) alkaline picrate technique. 50 cm^3 of distilled water was used to dissolve the 5.0 g ground sample after it was weighed. After an overnight stay, the cyanide extraction was filtered.

Determination of Steroid

Ten millilitre volumetric flasks were filled with one millilitre (1 ml) of the test extract of the steroid solution. Potassium hexacyanoferrate (iii) solution (0.5%w/v, 0.5ml) was added after 1 ml of Iron(iii) chloride ($FeCl_3$) (0.5%w/v, 2ml) and 2 ml of sulphuric acid (4N) were added. After 30 minutes of heating in a water bath kept at 70 ± 2°C with periodic shaking, the mixture was diluted with distilled water to the appropriate level. At 780 nm, the absorbance was measured in relation to the reagent bank (Yang *et al.,* 2010).

Determination of Terpenoid

Five millilitres (5 ml) of the plant sample extract were taken, and 2 ml of chloroform and 3 ml of 30% sulphuric acid (H_2SO_4) were thoroughly mixed. The mixture was then incubated in the water bath at 70°C for 5 minutes, allowed to cool, and the absorbance at 538 nm was measured (Pereira, 2023).

RESULTS AND DISCUSSION

This study investigated the *invitro* antimicrobial efficaciousness of *Datura stramonium* leaf extracts against *Helicobacter pylori*, a type of bacteria known to infect the stomach lining and be linked to a number of gastrointestinal disorders.

The efficacy of antibiotics

Table 1 showed the antibacterial properties of the antibiotic chloramphenicol and *Datura stramonium* leaf extracts against *Helicobacter pylori*. The ethanolic extract showed a zone of inhibition of 22.02 ± 0.02 mm at the highest dose of 500 mg/ml, the aqueous extract of 15.03 ± 0.03 mm, and the hexane extract of 17.03 ± 0.03 mm, in comparison to the reference medication, chloramphenicol, which showed a zone of inhibition of 25.03 ± 0.03 mm. These values dropped to 15.04 ± 0.04 mm, 13 ± 0.00 mm, and 15 ± 0.00 mm, respectively, when the concentration dropped to 250 mg/ml, with chloramphenicol displaying 18 ± 0.00 mm. The extracts from ethanol, water, and hexane revealed 12 ± 0.00 mm, 10 ± 0.00 mm, and 14.03 ± 0.03 mm at 125 mg/ml, respectively, while the extract from chloramphenicol showed 16.04 ± 0.04 mm. An inhibition of 9 ± 0.00 mm was seen in the ethanolic extract at 62.5 mg/ml; no inhibition was seen in the aqueous extract, 13.15 ± 0.02 mm in the hexane extract, and 15.05 ± 0.05 mm in the chloramphenicol extract. When the concentration was reduced to 31.25 mg/ml, the hexane extract was the only one to exhibit inhibition at 10 ± 0.00 mm. The ethanolic and aqueous extracts did not show any inhibition, whereas chloramphenicol showed 12 ± 0.00 mm. Only Chloramphenicol showed inhibition against the isolates at 10 ± 0.00 mm at the lowest dose of 15.63 mg/ml.

Table 1: Antimicrobial properties of the leaves extracts of *Datura stramonium* on *Helicobacter pylori*

Concentration (mg/ml)	Diameter zone of inhibition of extracts/ antibiotics (mm)			
	Ethanolic	**Aqueous**	**Hexane**	**Chloramphenicol**
500	22.02 ± 0.02	15.03 ± 0.03	17.03± 0.03	25.03 ± 0.03
250	15.04 ± 0.04	13 ± 0.00	15 ± 0.00	18 ± 0.00
125	12 ± 0.00	10 ± 0.00	14.03 ± 0.03	16.04 ± 0.04
62.5	9 ± 0.00	-	13.15 ± 0.02	15.05 ± 0.05
31.25	-	-	10 ± 0.00	12 ± 0.00
15.63	-	-	-	10 ± 0.00

Minimum inhibitory concentration and Minimum Bactericidal Concentration

The Minimum Bactericidal Concentration (MBC) is the lowest concentration of an antimicrobial agent that would wholly destroy the organism, even if it is still alive, whereas the Minimum Inhibitory Concentration is the lowest concentration that will inhibit or stop the growth of a bacterium.

Using ethanol, aqueous, and n-hexane as solvents, Table 2 below showed the antibacterial activities of *Datura stramonium* extracts against *H. pylori*, with an emphasis on the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). The ethanol extract of *Datura stramonium* demonstrated a moderate level of effectiveness, with a MIC of 125 mg/ml and an MBC of 500 mg/ml. The ethanol extract's MIC and MBC values were comparable by the N-hexane extract, while the aqueous extract's MIC was higher at 250 mg/ml and it exhibited no bactericidal activity.

Table 2: Minimum Inhibitory and Bactericidal concentrations of *Datura stramonium* against *H.pylori*

Bacterium	**Observations**					
	***Datura stramonium* leaves extracts**					
H.pylori	**Ethanol**		**Aqueous**		**Hexane**	
	MIC	MBC	MIC	MBC	MIC	MBC
	125	500	250	nil	125	500

Values are in mg/ml

Plant compound profiling

The nature and concentration of the bioactive molecules known as phytochemicals found in medicinal plants have been related to their therapeutic efficacy. A plant's diverse antimicrobial responses are prompted by a variety of substances. Various plant species and plant components contain these phytochemicals in large quantities. Table 3 and 4 shows the Trait oriented and Quantity- Specific plant compound profiling of Crude extracts *Datura stramonium*.

Table 3: Trait-oriented Plant compound profiling of crude extracts of *Datura stramonium*

Phytochemicals	**Observations *Datura stramonium***
Phenol	+++
Saponin	++
Tannin	+++
Alkaloids	+++
Flavonoids	+
Steroids	+++
Triterpenoids	++
Glycoside	+++
Reducing sugar	---
Proteins	---

+++ = Found in notable concentrations
++ = Found in moderate concentrations
+ = Found in trace concentrations
--- = Absent

Table 4: Quantity-Specific Plant compound profiling of crude extracts of *Datura stramonium*

Phytochemicals	**Observations *Datura stramonium* Value (mg/ml)**
Phenol	131.8 ± 0.03
Saponin	14.1 ± 0.02
Tannin	124.2 ± 0.01
Alkaloids	100.5 ± 0.00
Flavonoids	2.6 ± 0.02
Steroids	31.9 ± 0.01
Triterpenoids	37.9 ± 0.01
Glycoside	74.4 ± 0.04
Reducing sugar	0.00 ± 0.00
Proteins	0.00 ± 0.00

Values are means of triplicate

Discussion

Because of the therapeutic components that medicinal plants possess, using them to cure human diseases has been an ongoing tradition for millennia. Many compounds with established medicinal properties are produced by these plants, some of which have the ability to stop the spread of infections or eradicate them completely without having a major negative impact on host cells. These substances are thought to be excellent prospects for the creation of novel antibiotic medications (Bai *et al.,* 2020). This compares the *invitro* effectiveness of extracts from *stramonium* leaves extracts against *Helicobacter pylori*.

Table 1 lists the antibacterial properties of the antibiotic chloramphenicol and multiple *Datura stramonium* leaf extracts (ethanolic, aqueous, and hexane) against *Helicobacter pylori*. The zone of inhibition was used to calculate the activity in millimetres (mm). The ethanolic extract showed a zone of inhibition of 22.02 ± 0.02 mm at the highest dose of 500 mg/ml, the aqueous extract of 15.03 ± 0.03 mm, and the hexane extract of 17.03 ± 0.03 mm, in comparison to the reference medication, chloramphenicol, which showed a zone of inhibition of 25.03 ± 0.03 mm. These values dropped to 15.04 ± 0.04 mm, 13 ± 0.00 mm, and 15 ± 0.00 mm, respectively, when the concentration dropped to 250 mg/ml, with chloramphenicol displaying 18 ± 0.00 mm. The extracts from ethanol, water, and hexane revealed 12 ± 0.00 mm, 10 ± 0.00 mm, and 14.03 ± 0.03 mm at 125 mg/ml, respectively, while the extract from chloramphenicol showed 16.04 ± 0.04 mm. An inhibition of 9 ± 0.00 mm was seen in the ethanolic extract at 62.5 mg/ml; no inhibition was seen in the aqueous extract, 13.15 ± 0.02 mm in the hexane extract, and 15.05 ± 0.05 mm in the chloramphenicol extract. When the concentration was reduced to 31.25 mg/ml, the hexane extract was the only one to exhibit inhibition at 10 ± 0.00 mm. The ethanolic and aqueous extracts did not show any inhibition, whereas chloramphenicol showed 12 ± 0.00 mm. Only Chloramphenicol showed inhibition against the isolates at 10 ± 0.00 mm at the lowest dose of 15.63 mg/ml. According to this results, the ethanolic extract of *Datura stramonium* is the most efficient in treating *Helicobacter pylori* infections among the three extracts; which is also consistent with the findings of the study conducted by Gachande and Khilare (2013). There are multiple reasons for this increased effectiveness. As a polar solvent, ethanol can dissolve a variety of bioactive substances, including those with strong antibacterial effects, producing an extract that is more potent (Tarapatskyy *et al.,* 2021). Additionally, compared to other solvents, it extracts these beneficial substances more effectively because of its effectiveness in breaking through plant cell walls (Do *et al.,* 2014).

The plant extract's action on *H. pylori* reveals variations in distinct zones of inhibition, which illustrates how the extract's concentration affects its antimicrobial activity. When *Datura stramonium* was extracted using ethanol, the extract displayed the greatest zone of inhibition against *H. pylori* at 500 mg/ml, the lowest zone of inhibition, and the maximum concentration at 62.5 mg/ml.

The evaluation of the Minimum Bactericidal Concentration (MBC) and Minimum Inhibitory Concentration (MIC) of ethanolic, hexane, and aqueous extracts from *Datura stramonium*. This comparison offers valuable information regarding the extracts' efficacy against *H. pylori*. Contrary to the aqueous extract, which has a higher MIC of 250 mg/ml, the ethanolic and n-Hexane extracts of *Datura stramonium* have substantial antibacterial action against *H. pylori*, as demonstrated by their lower MIC values of 125 mg/ml. This implies that the extracts of ethanolic and n-hexane are more successful in suppressing the growth of *H. pylori*. The effectiveness of the ethanolic and n-Hexane extracts is further demonstrated by the MBC results. With an MBC of 500 mg/ml, both extracts are capable of eliminating *H. pylori* at this concentration. As an effective antibacterial treatment, however, the aqueous extract is limited because it does not exhibit bactericidal effects at any of the tested doses. There could be a number of reasons why *Datura stramonium's* aqueous extract lacks bactericidal properties against *H. pylori*. Firstly, the bioactive substances that give rise to bactericidal activity might not be efficiently extracted or preserved by water. Furthermore, it's possible that the aqueous extract's pH and chemical makeup are inappropriate for preserving the potency or stability of these substances. Finally, decreased efficacy may result from the principal antimicrobial drugs' decreased solubility in water as compared to alternative solvents like ethanol or N-hexane (Imarenezor *et al*., 2022).

The phytochemical makeup of the extracts in Table 3 and 4, shows both quantitative and qualitative of *Datura stramonium* leaf extract and provides important information on the bioactive substances that underlies the antimicrobial activities that have been reported. Phytochemical concentrations and presence in plants have been linked to their antibacterial action (Supriya *et al.,* 2019). Plants generally have higher inhibitory effects on the growth of Gram- Positive bacteria than Gram negative bacteria. As previously reported by (Selvadurai and Shanmugapandiyan, 2022). Crude extracts of *Datura stram onium* were discovered to contain a variety of phytochemicals, including alkaloids, tannins, phenolics, glycosides, steroids, triterpenoids, saponins, and flavonoids. The discovery of steroids in *Acacia mellifera* bark by (Veronica *et al.,* 2017) supports the theory that steroids are present in plant extracts. But different plant extracts may contain different amounts of steroids (Egbunu *et al.,* 2019).

Alkaloids and phenolics, which are substances with antibacterial qualities, were found in higher concentrations in *Datura stramonium* extract, supporting the findings of (Al-Snafi, 2017).

CONCLUSION

This study assessed the *Datura stramonium* extracts' antibacterial properties *in vitro* against *Helicobacter pylori* in three different forms: ethanol, aqueous, and n-hexane. The ethanol extract showed the strongest antibacterial activity of all the solvents studied, indicating that ethanol is a useful solvent for removing bioactive substances from *Datura stramonium* that have an effect on *H. pylori.* Given the increasing prevalence of antibiotic resistance, the ethanol extract's better performance underscores its potential for the development of natural therapeutic agents for the management of *H. pylori*-related infections.

Impact to knowledge

The study titled *In vitro* Evaluation of the Antibacterial Efficacy of *Datura stramonium* Leaf extract Against *Helicobacter pylori* demonstrated substantial implications for tackling a critical global health issue. The outcomes shows that *Datura stramonium* is a natural antibacterial agent that has promise as a substitute for conventional antibiotics, especially when it comes to treating infections caused by *H. pylori*. In light of the growing prevalence of antibiotic resistance, plant-based treatments provide creative and long-lasting remedies. The study connects traditional knowledge with contemporary scientific research by confirming the ethnomedical use of *Datura stramonium* for gastrointestinal ailments. Additionally, it establishes the framework for the isolation of bioactive substances that may result in the creation of innovative, low-cost treatment alternatives that target *H. pylori*. This result may help manage conditions like stomach cancer and peptic ulcers, particularly in regions with limited resources and high *H. pylori* prevalence. This has significant public health implications. *Datura stramonium's* potential for widespread use is further supported by its affordability and accessibility as a commonly available plant species.

RECOMMENDATION

To further our understanding of *Datura stramonium's* significance as an alternate ulcer treatment option, more research into the exact bioactive components responsible for its antibacterial activity against *H. pylori* isolates from ulcer patients and studies on their mechanisms of action are important.

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