



Original article

In – Vitro activity of *Desmostachya bipinnata* (L.) Stapf successive extracts against *Helicobacter pylori* clinical isolates

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ABSTRACT

Helicobacter pylori are well acknowledged as a major cause of gastrointestinal ailments and gastric cancers. Therefore, the present study aimed to investigate the potential *in vitro* activity of *Desmostachya bipinnata* against *H. pylori*, focusing on the determination of the most active extract responsible for the anti-helicobacter activity to produce new active drug from natural source.

Desmostachya bipinnata total alcohol and successive extracts were *in vitro* tested against *H. pylori*. All extracts showed promising anti *Helicobacter pylori* activities. The most effective extract was diethyl ether extract, it showed 75% growth inhibition of the clinical Isolates bacterial *Helicobacter pylori*, in addition it showed high count reduction on the selected organisms in the different concentrations used (2xMIC, MIC & ½ MIC) compared with the untreated controls as well as the other extracts (chloroform, ethyl acetate and n-butanol). The oral median lethal dose (LD₅₀) of the alcohol extract of the plant by doses up to 5000 mg/kg didn't showed any mortality or morbidity, in addition no side effects were recorded on both liver and kidney functions this means that the extract was safe for use.

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1. Introduction

Helicobacter pylori have been designated as critical organisms in the etiology of chronic gastritis; peptic ulcers (Megraud, 1993) and gastric cancers (NIH Consensus Development Panel, 1994; Mbulaiteye et al., 2009) and their suppression and eradication have been considered the gold standard therapy for infectious gastric diseases (Ibrahim et al., 2012). Many therapeutic agents are used for extermination of this bacterium, but the widespread use of these agents has increased resistance, failure and relapses among

the isolated strains of *H. pylori*. Therefore, the world is tending to go back to the use of natural products for the treatment of various diseases. Studies have suggested many natural plant extracts with anti-*H. pylori* activity, including chamomile flowers, coneflower herbs, peppermint leaves, thyme herbs and grapes as well as “Halfa grass” (Malm et al., 2015; Brown et al., 2009; James, 2011).

“Halfa grass” or *Desmostachya bipinnata* is included in the family *Gramineae*. This family comprises more than 660 genera and 10,000 species. It is well known for its great economic and medicinal importance because it includes all cereals, bamboos and sugar cane. There are many medicinal activities of *Gramineae* species such as astringent and in treatment of wound, anti-emetic, diuretic (Shrestha, 2011), and in treatment of eye problems (Bolus, 2000). *Desmostachya bipinnata* is a native plant in northeast and west tropical, and northern Africa and countries in the Middle East, and temperate and tropical Asia. Commonly known in English by the names ‘Halfa grass’, Big cord grass, and Salt reed-grass (James, 2011).

In folk medicine, it has been used to treat many diseases. Researchers proved that this plant has some activities as a diuretic,

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antidiarrheal (Medha et al., 2010), analgesic, antipyretic and has an anti-inflammatory effect (Panda et al., 2009). It was also suggested to have hepatoprotective ability (Rahate et al., 2015), help against dysentery, menorrhagia, jaundice, (Joshi, 2003) and has antioxidant effect (Golla & Bhimathati, 2014) as well as anti-ulcerogenic properties (Awad et al., 2008).

Few reports have recognized this plant as a candidate that showed promise as a novel antimicrobial agent (Hashmi & Rashid, 2001; James, 2011; Joshi et al., 2017). These reports and others have encouraged the current investigation. Therefore, the present study aimed to investigate the potential *in vitro* activity of *Desmostachya bipinnata* against *H. pylori*, focusing on the determination of the most active extract responsible for the anti-helicobacter activity to produce new active drug from natural source.

2. Material and methods

2.1. Plant material

Aerial parts of *Desmostachya bipinnata* (L.) Stapf were collected during flowering stage in March 2013 from Gazan territory, South west Saudi Arabia, the sample was identified by Dr. Jacob Thomas; Assistant Professor of taxonomy, Botany and Microbiology Department, Faculty of Science, King Saud University, Specimen was kept in the herbarium of botany Department, KS, KSA and were compared with published (Migahid, 1996). Samples of the aerial parts were air dried in shade, reduced to fine powder and kept for phytochemical and biological investigation.

2.2. Phytochemical screening, extraction and isolation

For determination of the phytochemical active constituents, the powder of *Desmostachya bipinnata* was subjected to screening according to the published methods (Ayoola et al., 2008).

Dried aerial parts of *Desmostachya bipinnata* (500 g) were extracted by percolation in ethanol (95%) at room temperature for two days. The ethanol extract was filtered and the residues were re-percolated as before (3 × 3 times). The combined filtrates were concentrated under reduced pressure to yield 80 g. The residue obtained was suspended in water (200 ml), filtered over a piece of cotton. The material obtained on top of the cotton piece (20 g) was symbolized as **D-1(The non-polar components)** and kept for further investigation. The aqueous layer which filtered off (**polar components**) were successively fractionated using: ether, chloroform, ethyl acetate and n-butanol respectively. Each extract was dried over anhydrous sodium sulphate and concentrated to yield 10, 8, 13 and 25 g. respectively. The dry extracts were kept for further investigation.

2.3. Anti – *H. Pylori* activity

2.3.1. Preparation of the extracts

The total methanol extract and the successive extracts (ether, chloroform, ethyl acetate and n-butanol) of *Desmostachya bipinnata* were suspended in DMSO (dimethyl sulfoxide) to be used on the selected strains. The antibiotic amoxicillin (Sigma Chemical Co., St. Louis, Mo.) was used as the standard control in every run.

2.3.2. Bacterial isolates

Twelve clinical yields of *Helicobacter pylori* were isolated from 19 biopsies received from patients diagnosed with gastritis or peptic ulcer at King Khalid University hospital, Riyadh, Saudi Arabia. Identification was carried out using Gram staining, urease, oxidase, and catalase tests. They were then stored in aliquots of 1 ml

glycerol-containing skimmed milk, following Han et al. method, at -70°C (Han et al., 1995) till used.

On use, the thawed bacterial growths were sub-cultured on blood agar supplemented with 5% defibrinated sheep blood (Bio-life) and *Helicobacter pylori* Selective Supplement (DENT, Oxoid, United Kingdom), incubated at 37°C in a microaerophilic atmosphere (5% O_2 , 10% CO_2 , and 85% N_2 ; Campy Gen; Unipath) for 3–5 days. The reference strains *H. pylori* ATCC 43504 and *Escherichia coli* ATCC 25922 were involved as controls with each run.

2.3.3. Antimicrobial screening

H. pylori inhibitory zone testing for the plant extracts selected were performed according to the modified method of Johnson and Christine (1995). Fresh microbial inocula were prepared from the selected strains and suspended in tubes containing sterile saline adjusting their turbidity to 2 McFarland (12×10^8 CFU/ml). The bacteria were then cultivated on the surface of Mueller-Hinton agar (Unipath S.p.A., Garbagnate Milanese, Milan, Italy) supplemented with 5% defibrinated sheep blood (Bio-life) and *Helicobacter pylori* Selective Supplement (DENT, Oxoid, United Kingdom). Wells sized 7-mm in diameter were punched on the plates with 30 μL of the prepared dissolved extracts incorporated in each well. DMSO was used as control in separate wells. The plates were permitted to diffused at 4°C for 2 h, then, incubated in microaerophilic conditions as mentioned before. The inhibition zones around each well were observed and their diameters were recorded.

2.3.4. Determination of the minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) was carried out by the broth microdilution assay (Hachem et al., 1996).

A total of 100 μL of Brain Heart Infusion broth supplemented with 10% defibrinated sheep blood inoculated with (12×10^8 CFU/ml) *Helicobacter pylori* (equal to 2 McFarland turbidity) and 100 μL of serial dilutions to reach final concentrations of 50, 25 > 50, 12.5 > 50, 6.25 > 50, 3.125 > 50, 1.5625 > 50, 0.78125 > 50 mg/mL. The standard drug, Amoxicillin, was diluted to the same concentrations. The microplate was incubated at 37°C under microaerophilic conditions in an atmosphere of 5–15% O_2 and 5–10% CO_2 , for 48–72 h. The MICs of each isolate were assessed visually as the lowest concentration of the extracts showing complete growth inhibition of the isolate as well as the reference strain. A positive control (containing inoculum without the tested extracts) and a negative control (containing the tested extracts without inoculum) were included on each microplate.

2.3.5. Time Kill-curve

The three most sensitive strains were selected and prepared in 100 μL of Brain Heart Infusion broth supplemented with 10% defibrinated sheep blood inoculated with (12×10^8 CFU/ml) *Helicobacter pylori* (equal to 2 McFarland turbidity). The ability of the most effective three extracts, in concentrations of MIC, $2 \times$ MIC and $\frac{1}{2}$ MIC, to inhibit the three growths was evaluated based on the plotting of time-death curves. Each concentration of the 3 tested extracts was tested against the selected yields and incubated for 1, 4 and 24 h at 37°C under microaerophilic conditions. Aliquots of the bacterial cultures were serially diluted and then plated on to MHA for colony counts. Parallel controls were carried out.

The effects of the extracts on the 3 *H. pylori* cell wall were also observed by the Scanning Electron Microscope following treatment by the extracts after 1, 4, and 24 h with MIC of each isolate.

2.4. Pharmacological study

2.4.1. Preparation of the plant extract

Dried aerial parts of *Desmostachya bipinnata* (L.) Stapf, were extracted as mentioned before in Section 2.2. The dried plant

extract was freshly suspended in distilled water just before administration.

2.4.2. Determination of median lethal dose (LD_{50})

LD_{50} of the ethanol extract was determined as described before (El-Meligy et al., 2017). For this purpose, 5 groups of 5 mature mail Swiss albino mice (23–25 g body weight) each were used. The tested extract was administered orally in doses of 200–400 mg/kg b.wt in addition to a group used as a control (given the solvent). Mice were kept under observation for 24 h during which the number of dead animals in each group was recorded.

2.5. Statistical analysis

Data were collected and analyzed statistically using Statistical Package for Social Sciences program (SPSS v21). The following tests were used in this study: mean, standard deviation, T test for independent samples, ANOV A test (analysis of variance). Significance levels: $P > 0.05$ insignificant, $P < 0.05$ significant and $P < 0.001$ highly significant.

3. Results and discussions

3.1. Phytochemical screening

Phytochemical screening of *Desmostachya bipinnata* (L.) showed the presence of the following groups: carbohydrates and/or glycosides, flavonoids, sterols and/or triterpenes, protein and/or amino acids, phenolic compounds and tannins while saponin, Anthraquinones, alkaloids, and cardinolides were absence in this plant.

3.2. Anti-*H. Pylori* activity

Twelve clinical yields of *Helicobacter pylori* were identified from 19 biopsies received from patients diagnosed with gastritis or peptic ulcer at King Khalid University hospital, Riyadh, Saudi Arabia.

On screening of the 5 prepared extracts by the cup diffusion method, the results showed susceptibility and inhibition zones for the different extracts. The most effective were ether extract (EE), total extract (TE) and *n*.butanol extract (BE) which showed inhibition zones in 9 (75%), 9 (75%) and 8 (66.7%) isolates respectively. These results were statistical significant when compared with the control wells containing the solvent alone (p value of 0.0003) (table 1).

MICs of the 3 most active extracts were assessed against the 12 selected isolates by broth microdilution method. The findings revealed that Total extract was the most effective extract against

the growths, though with no statistical significance, moreover, isolates 2, 3 and 7 were the most sensitive yields, which made them candidates for further evaluation (Table 2 & Fig. 1).

The 3 most sensitive isolates (2, 3 & 7 renamed as I, II & III) were selected and subjected to the effect of the most effective three extracts, in concentrations of MIC, $2 \times$ MIC and $\frac{1}{2}$ MIC, to inhibit the three growths. They were evaluated based on the plotting of time-death curves; each concentration of the 3 tested extracts was tested against the selected yields and incubated for 1, 4 and 24 h at 37 °C under microaerophilic conditions. There were statistical significant difference regarding time and concentrations when presented in means, $\frac{1}{2}$ MIC concentration when compared with MIC and $2 \times$ MIC of the selected strains p value were 0.04 and 0.000 respectively. Different time periods used showed also statistical significance; 1 h when compared with 4 h and 24 h, p value were 0.01 and 0.000 respectively. However, there was no statistical significant difference in-between the different extracts, except for, between total and *n*.butanol extract in their MIC for 24 h; the p value was 0.01 (Fig. 2).

The *in vitro* effectiveness of the extracts on the *3H. pylori*'s cell wall were also assessed using the Scanning Electron Microscope following treatment by the extracts after 1, 4, and 24 h with MIC of each isolate. The cell wall was indented and showed many convolutions after only one hour, after 4 h, it became more damaged to be more and more destructed at the 24 h time period (Fig. 3).

3.3. Determination of median lethal dose (LD_{50})

The oral median lethal dose (LD_{50}) of the alcohol extract of *Desmostachya bipinnata* (L.) Stapf by doses up to 5000 mg/kg didn't showed any mortality or morbidity. This means that, the extract was safe for use (Lorke 1983; Awaad Amani et al., 2015).

Table 2
MICs of the 3 selected extracts by broth micro-dilution assay.

Isolates	Extracts		
	Minimum inhibitory concentration MIC (mg/ml)		
	Ether extract	Total extract	<i>n</i> .Butanol
1	25	50	12.5
2	12.5	12.5	>50
3	25	6.25	6.25
4	25	12.5	>50
5	50	50	12.5
6	12.5	25	>50
7	12.5	6.25	>50
8	25	25	>50
9	>50	>50	>50
10	>50	>50	>50
11	>50	>50	>50
12	>50	>50	>50

Table 1
Analysis of anti-*Helicobacter pylori* activity of plant extracts by cup diffusion method.

Isolates	Extracts				
	Inhibition zone in (mm)				
	Total alcohol	Non polar	Ether	Ethyl acetate	<i>n</i> .butanol
1	13	9	14	10	17
2	18	10	16	≥ 7	13
3	17	15	20	13	18
4	15	≥ 7	16	14	15
5	12	13	10	≥ 7	12
6	13	≥ 7	11	≥ 7	15
7	18	13	14	13	13
8	13	≥ 7	12	≥ 7	11
9	≥ 7	≥ 7	11	≥ 7	≥ 7
10	10	≥ 7	≥ 7	≥ 7	≥ 7
11	≥ 7	≥ 7	≥ 7	≥ 7	≥ 7
12	≥ 7	≥ 7	≥ 7	≥ 7	≥ 7

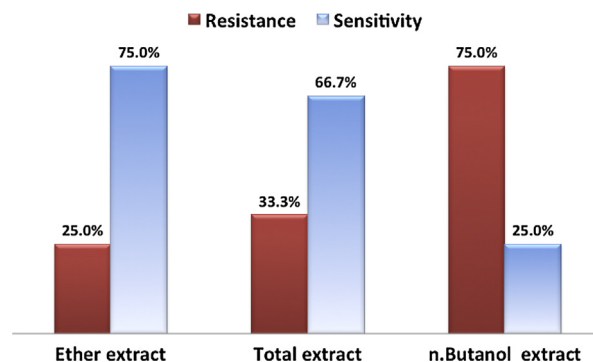


Fig. 1. Comparative *in vitro* MIC activities of different extracts against 12 clinical *H. pylori* isolates.

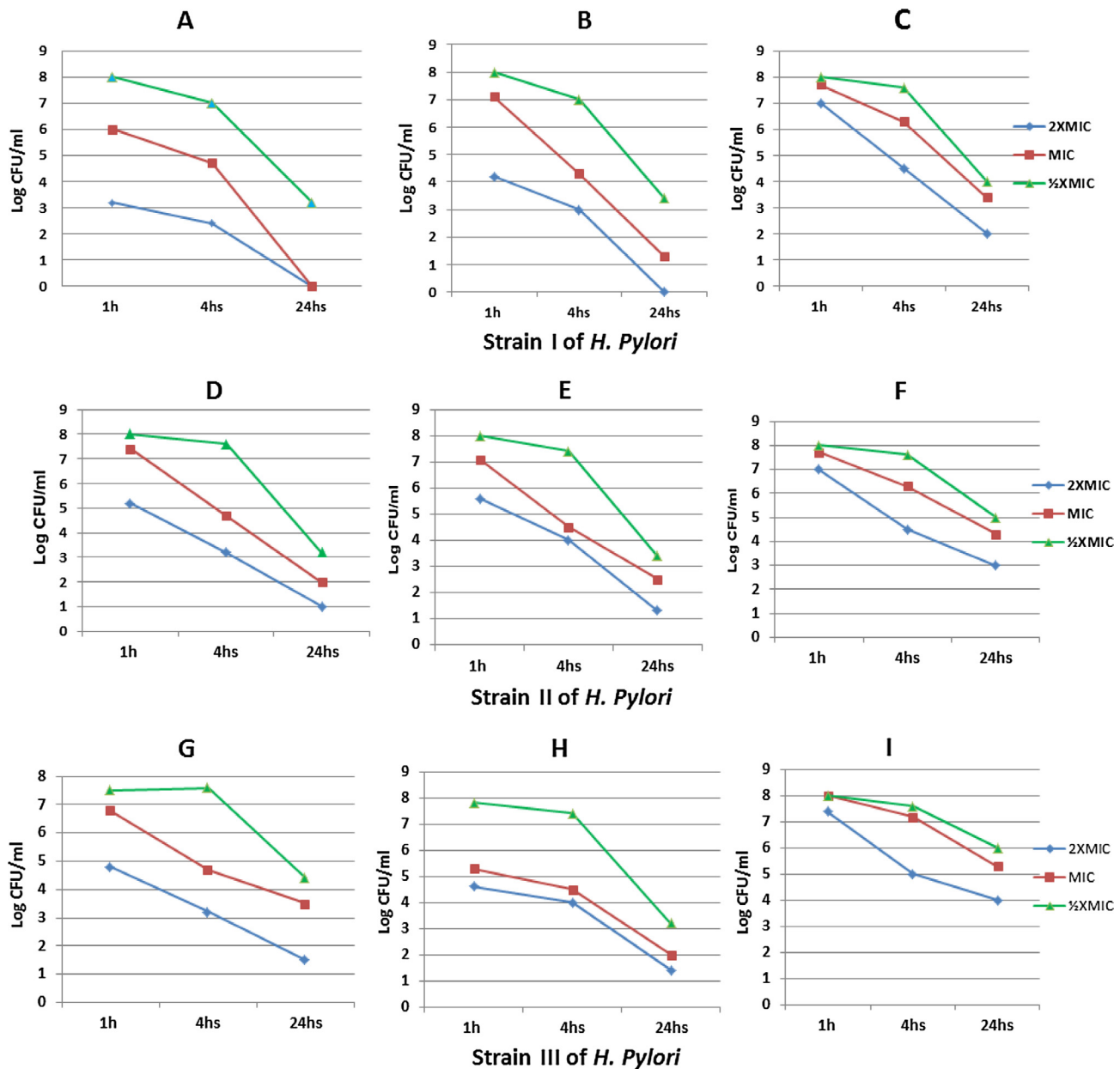


Fig. 2. A, D & G: the time dependent effect of (2 × MIC, MIC & 1/2 MIC) of diethyl ether extract on the three selected isolates at 1, 4, 24 h exposure time. B, E & H: the time dependent effect of (2xMIC, MIC & 1/2 MIC) of the total extract on the three selected isolates at 1, 4, 24 h exposure time. C, F & I: the time dependent effect of (2xMIC, MIC & 1/2 MIC) of *n*.butanol extract on the three selected isolates at 1, 4, 24 h exposure time.

The non-toxic nature of *Desmostachya bipinnata* (L) Stapf ethanol extract in acute toxicity study is well supported by the biochemical data following 35 consecutive days of oral administration of the total alcohol extract to rats in a dose of 500 mg/kg which neither show any significant effect on the activity of **ALT** and **AST**, nor any significant changes in the mean values of urea and creatinine in rat's sera compared to controls (Table 3). The alcohol extract fractions of *Desmostachya bipennata* (L.) Stapf are therefore, neither hepatotoxic nor nephrotoxic in rats.

3.4. Antihelicopacter

The relationship between *Helicobacter pylori* and acquired resistance to various drugs from conventional therapy is of worldwide concern. Several global agreement assemblies have been con-

ducted to ensure that therapeutic guidelines are constantly revised on various issues involving the controlling of infections (Malfertheiner et al., 2012). With antibiotic resistance reaching a predicament in many hospitals around the world and the growing resistance rate disturbing the communities, there is a crucial need to reestablish the armory of new antimicrobial agents (Tim Cushnie and Lamb, 2011). Furthermore, the prospects for incompletion to treatment are large, mainly, due to side effects or recurrence of infection. Therefore, it was imperative to search for new remedies with anti-*Helicobacter pylori* action. Currently, plants are viewed as the main source for the discovery of new compounds (Cogo et al., 2010; Peek & Crabtree, 2006).

For centuries, many medicinal plants were employed to treat various gastrointestinal tract illnesses. They contain a lot of biologically active compounds that may display potential antimicrobial

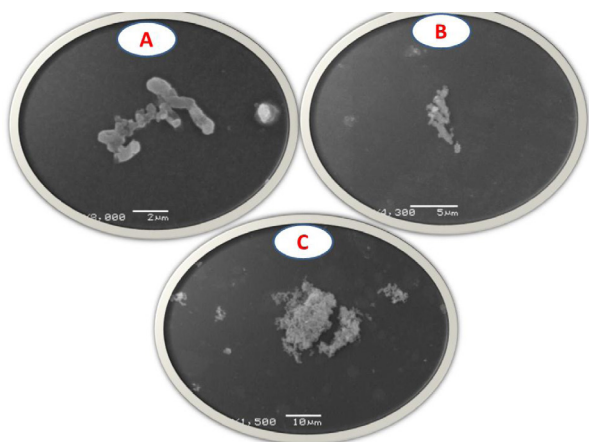


Fig. 3. Scanning electron microscopy images of *H. pylori* following treatment by diethyl ether extract after 1, 4, and 24 h with MIC of each isolate. A: the isolate after 1 h of treatment, B: the organism after 4 h, C: the organism after 24 h.

Table 3
Effect of *Desmostachya bipinnata* (L.) Stapf extract on liver and kidney functions of rats.

Groups	ALT(U/l)	AST(U/l)	Blood urea (mg/dl)	Serum creatinine (mg/dl)
Control	4.70 ± 0.47	4.90 ± 0.27	44.60 ± 1.26	0.79 ± 0.03
<i>Desmostachya bipinnata</i> (1000 mg/kg)	4.80 ± 0.02	4.80 ± 0.50	45.00 ± 0.05	0.80 ± 0.02

properties, including anti-*H. pylori* activity. Plants with potent anti-*H. pylori* effects were found to belong to several families (Lang and Buchbauer, 2012; Safavi et al., 2014)

The anti-*H. pylori* activity of plant extracts is studied *in vitro* usually by way of the disc or cup diffusion method or the microdilution method. The latter is recommended by the Clinical and Laboratory Standards Institute (CLSI, 2006; Ramadan & Safwat, 2009) in and the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2003) for determining MIC of antimicrobial substances (Wiegand et al., 2008).

In the current study, dried aerial parts of *Desmostachya bipinnata* (L.) Stapf were extracted by percolation in ethanol (95%). Total methanol extract and the successive extracts (ether, chloroform, ethyl acetate and n-butanol) were prepared and their anti-helicobacter effects were evaluated. The most effective extracts were ether extract (EE) and total extract (TE) followed by n-butanol extract (BE), which showed evident inhibition zones in 9 (75%), 9 (75%) and 8 (66.7%) isolates respectively (*p* value of 0.0003) (Table 1). MICs of the 3 most active extracts were assessed against the 12 selected isolates by broth microdilution method. The findings revealed that TE was the most effective extract against the growths. These results were comparable to the findings of Hashmi & Rashid (2001); James (2011) and Joshi et al. (2017), who found anti-bacterial activity of the extracts of the investigated plant, nevertheless, the tested parts in these reports were the roots.

The 3 most sensitive isolates were selected and subjected to the effect of the most effective three extracts, in concentrations of MIC, 2 × MIC and ½MIC, to inhibit the selected 3 yields. They were evaluated based on the plotting of time-death curves; each concentration of the 3 tested extracts was tested and incubated for 1, 4 and 24 h. There were statistical significant difference regarding time and concentrations when presented in means, ½MIC concentration when compared with MIC and 2 × MIC of the selected strains *p*

value were 0.04 and 0.000 respectively. Different time periods used showed also statistical significance; 1 h when compared with 4 h and 24 h, *p* value were 0.01 and 0.000 respectively (Fig. 2). The *in vitro* effectiveness of the extracts on the 3*H. pylori*'s cell wall were also assessed using the Scanning Electron Microscope following treatment by the extracts after 1, 4, and 24 h with MIC of each isolate. The cell wall was indented and showed many convolutions after only one hour, after 4 h, it became more damaged to be more and more destructed at the 24 h time period (Fig. 3). Further studies should be performed to confirm the activity of these extracts against more clinical *H. pylori* isolates, especially those showing drug resistance, and to study possible other mechanisms of their anti-*H. pylori* activity.

4. Conclusion

The present findings suggest that *Desmostachya bipinnata* (L.) Stapf aerial parts can be considered as a potential natural medicine. It has promising bioactivity against *H. pylori*, and has the ability for being used safely in prophylaxis or as an enhancing agent in *H. pylori* infection treatment.

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