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Anti-*Helicobacter pylori,* proton pump inhibitory and antioxidant properties of selected dietary/medicinal plants

Belagihalli Manjegowda Srikanta[1], Shylaja Mallaiah Dharmesh*[1]

*Corresponding author:

Shylaja Mallaiah Dharmesh

[1]Central Food Technological Research Institute (CFTRI), CSIR Laboratory, Mysore- 570 020, Karnataka, India.

Abstract

In recent days, emphasis has been on the use of edible sources for maintenance of health. *H. pylori* being a main causative organism of gastric cancer and ulcers, many researchers are working in this area to overcome the problem. The plants selected for the current study have application in traditional medicine for the treatment of gastrointestinal diseases. The studies provide the scientific evidence to antiulcer potential of medicinal/dietary plants of ethnopharmacological importance and categorize them based on multi-potent antiulcer activity. In the current study anti-*Helicobacter pylori,* H^+, K^+ -ATPase inhibitory and antioxidant activity of aqueous extracts of 18 different plants were investigated. The overall antiulcer potency of selected plants was calculated as antiulcer index (AUI) based on these *in vitro* antiulcer activities and categorized the plants into 4 groups based on their AUI. Overall study revealed *Decalepis hamiltonii* as very good (AUI 81) followed by *Nigella sativa, Coscinium fenestratum* and *Zingiber officinale* as good source (AUI 61-68) for antiulcer therapy. Among the rest, 9 plants were categorized as moderate with AUI of 41 to 59 and 6 plants as poor sources with AUI of 20 to 37. Thus the results of the current study contribute towards validation of the traditional use of selected plant sources against gastrointestinal disorders.

Keywords: Helicobacter pylori; H+, K+ -ATPase; Antioxidant; Gastric ulcer; Antiulcer index.

Introduction

Before the 19th century, peptic ulceration was uncommon, be it in East or West. Recently it became a common global problem because of change in life style [1]. A gastric ulcer is erosion in the lining of the stomach, which can lead to hemorrhage, perforation, obstruction and death. It develops due to stress, nonsteroidal anti-inflammatory drugs, ethanol consumption and *Helicobacter pylori* infection, where increased acid secretion is known to aggravate the ulcer and delays wound healing [2-4]. Among various mechanisms involved in the formation of gastric lesions, free radicals generated during stress and infection are the major causative factors for the gastric lesion through oxidative damage [5-6]. Thus the best approach to control gastric ulcer would be to inhibit oxidative damage, acid secretion and *H. pylori* infections in addition to re-enforcing integrity of gastric mucosal barrier [7-8].

The mechanism of free radical mediated damage during ulcer involves lipid peroxidation, which destroys cell membranes with the release of intracellular components, such as lysosomal enzymes, leading to further tissue damage. These free radicals also promote mucosal damage by causing degradation of the epithelial basement membrane components, alteration of the cell metabolism, decreasing the viscosity of mucous gel and DNA damage leading to gastric lesions [9].

Free radicals were also found to cause damage of parietal cells which harbors the rate limiting enzyme H^+, K^+ -ATPase or proton pump that participate in the final step of acid secretion [10] and all the peripheral parietal cell stimuli converge to activate it. Therefore, blocking the final process in hydrogen ion secretion also has been known to reduce acid secretion. Proton pump inhibitors were therefore designed by various investigators to provide faster onset of action in the control of acid secretion and hence greater relief of symptoms and increased healing rates [11].

It is important to note here that although these synthetic proton pump blockers worked effectively in blocking the acid secretion and acid induced ulcerations, alternative natural compounds/sources were always on hunt due to side effects of available synthetic proton pump blockers [12]. Another major factor needed attention was a control measures for *H. pylori* a major ulcerogen causing 70% of ulcers globally. *H. pylori* is a Gram-negative, helical bacteria found to colonize in human gastric epithelium [13].

In addition to gastric ulcers, *H. pylori* also found to associate with the development of gastric adenocarcinomas [14], gastric lymphoma and MALT lymphoma [15]. Eradication of *H. pylori* thus

was believed to contribute to the treatment and prevention of these diseases. It is possible that *H. pylori* inhibitors accelerate ulcer healing and may reduce relapse of the disease. Currently new triple therapies consisting of antibiotics and proton pump inhibitors show eradication rates much better than the individual drugs. However, problems arise due to *H. pylori* rapidly acquiring resistance to traditional antibiotics, in addition to side effects posed by synthetic proton pump blockers and mucoprotectants.

Thus the attention of clinical microbiologists now been focused on antimicrobial plant / dietary extracts. It is thought that first, it is very likely that these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by physicians; second, the public is becoming increasingly aware of problems with the over prescription and misuse of traditional antibiotics [16]. Therefore drugs of plant / dietary origin are gaining popularity and are being investigated for a number of disorders, including gastric ulcer [17].

Plant derived phenolic compounds have numerous beneficial properties by virtue of their ability to act as antioxidants, anticarcinogens, antimutagens and antiglycemic agents in addition to act as antimicrobial agents against human pathogens [18-19]. Further, plants were found to be the richest source of phenolic antioxidant molecules and have a long term history of their usages as antioxidants for different purposes including food preservation and prevention of health affecting complications, life protection, life extension etc. Since traditional medicine systems for treating gastric disturbances mainly depend on plant natural products, current study has been therefore aimed at undertaking the screening of traditionally known plant / dietary sources for precise determination of antiulcer potential. Potential ulcer preventive / curative sources would be arrived by determining the ability of these sources in inhibiting H^+, K^+ -ATPase enzyme and *H. pylori* growth followed by antioxidant abilities *in vitro.*

Materials and Methods

Materials

Agarose, gallic acid, and 1,1-diphenyl-2-picryl hydrazyl (DPPH), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), amoxicillin, Dimethyl sulphoxide (DMSO), Fetal bovine serum (FBS) and PCR reagents were obtained from Sigma Chemical Co.(St. Louis, MO). DNA isolation kit was purchased from Genei, India. Folin-Ciocalteu reagent, ferric chloride, trichloroacetic acid, sodium carbonate and ferrous sulphate were of the highest quality purchased from Qualigens Fine Chemicals (Mumbai, India). Ham's F12 nutrient mixture from Himedia (Mumbai, India) and Sterile syringe filters purchased from Millipore (Bangalore, India).

Isolation of *H. pylori*

H. pylori was isolated from endoscopic samples of gastric ulcer patients from KCDC (Karnataka Cardio Diagnostic Centre, Mysore, India) and cultured on Ham's F-12 nutrient agar medium with 5% FBS at 37 ^{0}C for 3 days in a microaerophilic condition with 5% CO_2 and 100% humidity [20]. *H. pylori* culture was confirmed by specific tests such as urease, catalase, oxidase, gram staining, colony characteristics and morphology by scanning electron microscopy as described earlier [21]. In addition, confirmation of virulence of *H. pylori* was performed by PCR amplification of Vaculating gene A (vacA) of *H. pylori* as described previously [22] , in order to confirm that the isolated strain has the ability to cause host cell vacuolation and ulcerations. The amplification of VacA gene was performed using VacA specific primers, forward 5' GTCAGCATCACACCGCAAC and 3', Reverse 5' CTGCTTGAATGCGCCAAAC 3'.

Preparation of plant extracts

Eighteen plant sources were selected based on ethnobotanical information. We have selected a wide range of plant sources including leaf, stem, root (rhizome), seeds and fruit peel as shown in Table 1 based on their traditional use as either food or medicine. The plant materials were collected either from local vendor at Devaraja market, Mysore or local places around Mysore, Karnataka state, India and identified with the help of flora [23] and also by referring them to the herbarium collections maintained at Department of Botany, Manasagangotri, University of Mysore, Mysore. For preparation of plant extracts, the plant materials were shade dried and powdered. 10 g of each powdered substance was dissolved in 100 mL of distilled, boiling water and stirred for 20 min. The extracts were filtered through muslin cloth and centrifuged at 2000 *g*. The supernatants were directly used for estimation of total phenols, H^+, K^+ -ATPase inhibition and antioxidant assays. For *H. pylori* inhibition studies, the pH of the supernatants were neutralized and further filtered through 0.22 µm sterile filters. All extracts were stored in the dark at –20 ^{0}C until use.

Estimation of total phenol content

The Folin-Ciocalteu reagent assay was used to determine the total phenolic content [24]. Known volume of extract was added and made up to 1 mL with water, 1 mL of Folin-Ciocalteu reagent previously diluted with distilled water (1:2 v/v) and 2 mL of 10% sodium carbonate solution in distilled water was added, mixed in a cyclo mixer. The absorbance was measured at 765 nm using a Shimadzu UV-Visible spectrophotometer (Shimadzu UV-160 spectrophotometer) after incubation for 2 h at room temperature. Gallic acid at concentration range of 5 to 25 µg/mL was used as a standard for the calibration curve. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligram per gram sample.

Antioxidant assays

The antioxidant activity was determined by free radical scavenging and reducing power assays as described earlier [21]. Free radical scavenging ability of extracts was determined employing 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay [25]. An aliquot of extract was added to 3 mL of 0.004% methanol solution of DPPH. The mixture was shaken vigorously and left to stand for 20 min at room temperature in the dark. The absorbance

of the resulting solution was measured spectrophotometrically at 517 nm. The activity was calculated in terms of phenolics of GAE required to scavenge 50% of DPPH free radical and expressed as IC_{50} values as µg GAE/mL.

The reducing power was determined according to the method of Yen and Chen [26]. The plant extracts were made up to 500 µL with 0.2 M phosphate buffer, pH 6.6 and added 500 µL of 1% potassium ferricyanide and incubated at 50 ^{0}C for 20 min. An equal volume of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 g for 10 min. The upper layer of solution was mixed with equal volume of distilled water and 300 µL of 0.1% $FeCl_3$ was added and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. The activity was expressed as absorbance units/mg GAE.

Table 1: List of plants used in the study

Scientific name	Common English name	Local name (Kannada)	Family	Part used
Alpinia galanga (L.) Willd.	Greater galangal	Rasmi	Zingiberaceae	Rhizome
Alternanthera sessilis DC.	Sessile joyweed	Honagone	Amaranthaceae	Leaf
Amorphophallus sylvaticus Roxb.	Amorphophalus	Suvarna gedde	Araceae	Tuber
Asparagus racemosus Willd.	Asparagus	Shathavari	Liliaceae	Root
Basella alba L.	Indian spinach	Basale	Basellaceae	Leaf
Basella alba L.	Indian spinach	Basale	Basellaceae	Stem
Cassine glauca Kuntze.	Ceylon tree	Mookurichi	Celastraceae	Leaf
Celosia argentea L.	Silver cockscomb	Anne	Amaranthaceae	Leaf
Cocculus hirsutus L.	Broom creeper	Dagadi balli	Menispermaceae	Leaf
Coleus aromaticus Benth.	Coleus root	Dodda pathre	Lamiaceae	Root
Coriandrum sativum L.	Coriander	Kothambari	Apiaceae	Leaf
Coscinium fenestratum Gaertn.	False calumba root	Marada arisina	Menispermaceae	Stem
Decalepis hamiltonii Wight & Arn.	Swallow root	Makali beru	Asclepiadaceae	Root
Murraya koenigii L.	Curry leaf	Karibevu	Rutaceae	Leaf
Nigella sativa L.	Black cumin (Fennel)	Kari geerige	Ranunculaceae	Seed
Passiflora edulis Sims.	Passion fruit	-	Passifloraceae	Peel
Solanum nigrum L.	Black nightshade	Ganike	Solanaceae	Leaf
Trigonella foenum-greacum L.	Fenugreek	Menthya	Fabaceae	Leaf
Zingiber officinale Rosc.	Ginger	Shunti	Zingiberaceae	Rhizome

Inhibition of H^+, K^+ -ATPase

The H^+, K^+ -ATPase enzyme of parietal cell was prepared from sheep stomach, as described earlier [21]. Briefly, the fundic mucosa of stomach was cut off and the inner layer was scraped off for parietal cells, homogenized in 16 mM Tris buffer (pH 7.4) and centrifuged at 6000 *g* for 10 min. The supernatant (enzyme extract) was used for the assay. Protein content was determined according to Bradford's method using bovine serum albumin (BSA) as standard.The enzyme extract (350 µg/mL) was incubated with different concentrations of plant extracts / bioactives in a reaction mixture containing 16 mM Tris buffer (pH 6.5) and the reaction was initiated by adding the substrate 2 mM ATP, in addition to 2 mM $MgCl_2$ and 10 mM KCl. After 30 min of incubation at 37 ^{0}C, the reaction was stopped by the addition of reaction mixture containing 4.5% ammonium molybdate and 60% perchloric acid. Inorganic phosphate formed was measured spectrophotometrically at 400 nm. Percent inhibition of enzyme activity was calculated and expressed as IC_{50} values, where IC_{50} is defined as amount of GAE of extract needed to inhibit 50% of enzyme activity. Lansoprazole was used as a known proton pump inhibitor.

Inhibition of *H. pylori* – Viable colony count method

Bactericidal activity of the plant extracts was determined by a viable colony count method [27]. One hundred microliters of a suspension of 10^8 colony forming units (CFU)/mL was added to 900 µL of plant extract and incubated for 60 min at 37 ^{0}C. The control consisted of *H. pylori* incubated with sterile distilled water. After incubation, serial 10-fold dilutions were made, and 100 µL of each dilution was plated onto Ham's F12 nutrient agar. These plates were incubated under microaerophilic conditions for 3 days, and colonies were counted. All experiments were performed three times. The effectiveness of the plants at killing *H. pylori* was expressed as percentage inhibition of colony growth (i.e. percentage of bacteria killed) compared to that of control.

Scanning electron microscopic (SEM) studies

H. pylori was incubated with different extracts as described in viable colony count method for 1 h. Amoxicillin was used as standard *H. pylori* inhibitor and the cells without inhibitor were taken as control. After incubation, cells were fixed with 2% glutaraldehyde and dehydrated with series of ethanol treatments and examined by SEM (Model No. LEO 425 VP, Electron microscopy LTD, Cambridge, UK) with an acceleration voltage of 20 KV. Multiple fields of visions were viewed at different magnifications [28].

Determination of antiulcer index

Aqueous extracts of the selected plant sources contain different quantities of phenolic compounds. These extracts possessed differential abilities for either H^+, K^+ -ATPase inhibition, *H. pylori* inhibition or antioxidant activities. The percent relative activity was calculated for each activity by considering the more potent value as 100% as described earlier [29]. Mean value of these percent relative activities for each plant were calculated and indicated as antiulcer index (AUI) as shown in Table 4. Antiulcer index has been further considered to categorize very good, good, moderate and poor sources.

Statistical analysis

All the experiments were carried out in triplicate (n = 3) and the results are expressed as mean ± standard deviation (SD).

Results

Inhibition of *H. pylori*

H. pylori isolated from biopsy sample of gastric ulcer patient was confirmed by different tests as described above. The virulence/pathogenic strain was further confirmed by PCR amplification of VacA gene, specific to pathogenic strain of *H. pylori*. It showed amplification of ~700 bp DNA of VacA gene similar to our previous observation [22]. The *H. pylori* culture was further used for inhibitory studies. We have selected a wide range of plant sources including leaf, stem, root (rhizome), seeds and fruit peel as shown in Table 1 based on their traditional medicinal value. Inhibitory property of plant extracts against *H. pylori* growth was analysed by colony count inhibition method. The results as shown in Table 2 indicate a varied potency among different plant extracts. *A. racemosus, N. sativa, C. glauca, C. fenestratum, C. aromaticus, C. hirsutus, Z. officinale* and *D. hamiltonii* showed more than 90% inhibition of *H. pylori* growth. *A. galanga, M. koenigii, T. foenum-greacum* and *P. edulis* showed moderate level of inhibition (~50 - 70%). The other plants showed very poor activity (less than 30%) against *H. pylori.*

Further the H. pylori killing effect of these extracts were analysed by SEM after treating H. pylori with plant extracts for 1 h. The SEM observations were correlated with the results of colony count inhibition assay. The plant extracts which showed more than 90% inhibition of H. pylori colony were effective also in producing marked changes in the morphology of bacterial cells as shown in Fig 1. These plant extracts modifies the H. pylori to coccoid shape, which appear to be inactive form. We have also observed clumping of cells, lysis of bacterial cell wall and leaking of cell contents during the treatment with plant extracts suggesting potent H. pylori inhibitory effect. These morphological changes were minimized in H. pylori treated with extracts, which showed moderate activity in colony count inhibition, they could induce only coccoid shape but there was no lytic effect observed. Similarly the extracts which showed poor activity in colony count inhibition method were unable to induce any morphological changes in H. pylori.

Table 2: Anti-*H. pylori* and proton pump inhibitory effects

Plants	% *H. pylori* inhibition	ATPase inhibition IC_{50} (µg GAE/mL)
Alpinia galanga	59 ± 4.1	34.62 ± 4.2
Alternanthera sessilis	25 ± 3.2	88.86 ± 7.9
Amorphophallus sylvaticus	27 ± 4.2	15.91 ± 2.0
Asparagus racemosus	93 ± 8.0	45.03 ± 5.1
Basella alba (leaf)	24 ± 4.0	29.08 ± 2.3
Basella alba (stem)	19 ± 2.1	45.11 ± 5.0
Cassine glauca	98 ± 6.5	280.25 ± 10.9
Celosia argentea	18 ± 2.0	89.62 ± 9.0
Cocculus hirsutus	94 ± 11.4	69.64 ± 6.9
Coleus aromaticus	93 ± 8.4	206.48 ± 18.5
Coriandrum sativum	34 ± 4.0	42.29 ± 3.6
Coscinium fenestratum	97 ± 5.5	13.20 ± 1.8
Decalepis hamiltonii	97 ± 9.9	29.45 ± 3.0
Murraya koenigii	69 ± 9.3	88.67 ± 6.9
Nigella sativa	97 ± 10.2	37.52 ± 3.0
Passiflora edulis	65 ± 6.9	22.49 ± 3.0
Solanum nigrum	23 ± 3.4	25.83 ± 2.5
Trigonella foenum-greacum	68 ± 8.2	18.64 ± 2.1
Zingiber officinale	93 ± 10.4	14.58 ± 1.9

Bactericidal activity of plants against *H. pylori*, determined using the viable colony count method. '% inhibition of growth' indicates the percentage of bacteria that were killed by the plants.

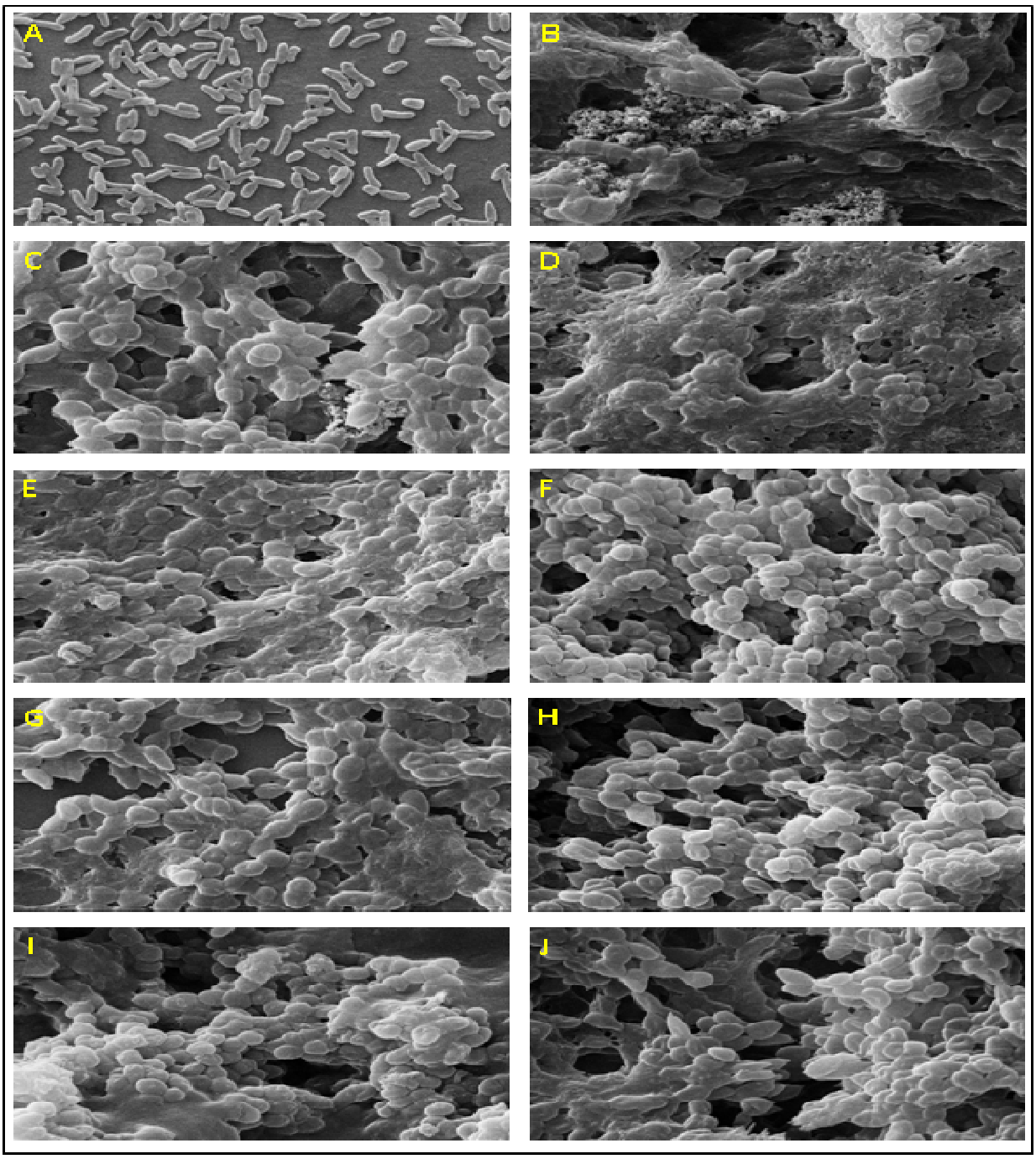

Fig 1. Scanning electron microscopic pictures of *H. pylori* treated with plant extracts exhibiting good *H. pylori* inhibitory activity: **SEM pictures of *H. pylori* at 10K magnification:** A – **Control,** B- **Amoxicillin,** C- ***A. racemosus***, D- ***N. sativa***, E- ***C. glauca***, F- ***C. fenestratum***, G- ***C. aromaticus***, H- ***C. hirsutus***, I- ***Z. officinale***, J- ***D. hamiltonii***. **The *H. pylori* inhibitory effect of plant extracts and amoxicillin were evidenced by change in morphology to cocci shape and lysed cells of *H. pylori*.**

Inhibition of H+, K+ -ATPase enzyme

All the extracts except *A. sessilis, C. glauca, C. argentea and C. aromaticus* inhibited the H^+, K^+ -ATPase with variable potency. The potency of the extracts determined as the concentration needed to inhibit 50% (IC_{50}) of the activity of the enzyme is presented in Table 2. *A. sylvaticus, C. fenestratum, Z. officinale, T. foenum-greacum, D. hamiltonii and S. nigrum* exhibited a potential inhibition of H^+, K^+ -ATPase activity with the IC_{50} values ranging from 13 to 29 µg GAE/mL. *A. galanga, A. racemosus, B. alba, N. sativa* and *C. sativum* exhibited a moderate inhibition of ATPase enzyme activity with IC_{50} values of 34 – 47 µg GAE/mL. *M. koenigii* and *C. hirsutus* exhibited a poor activity, whereas *A. sessilis, C. glauca, C. argentea* and *C. aromaticus* were unable to inhibit H^+, K^+ -ATPase activity even at 80 µg/mL concentration suggesting poorer H^+, K^+ -ATPase inhibitory or antiulcer effect (Table 2 & 4).

Phenol content and antioxidant potential

Wide range of total phenol concentrations were found among the selected 18 different plant sources (Table 3). The values varied from 1.97 to 44 mg GAE/g as measured by the Folin–Ciocalteau method. *C. glauca, M. koenigii, C. hirsutus* and *D. hamiltonii* showed higher level (23 – 44 mg GAE/g) of phenolics followed by medium level (12 – 18 mg GAE/g) of phenolics in *S. nigrum, Z. officinale, C. sativum, C. argentea* and *N. sativa.* Remaining sources possessed lesser amount of phenolic content (less than 10 mg GAE/g). However contribution to activity from them can still be significant.

Antioxidant potency tested by DPPH-free radical scavenging and reducing power assays is shown in Table 3 & 4. Good antioxidant activity was exhibited by A. galanga, N. sativa, C. aromaticus, C. hirsutus and D. hamiltonii extracts. They showed IC_{50} values range between 0.95 – 3.07 µg GAE/mL for DPPH-free radical scavenging effect. Also they exhibited potent reducing power with absorbance units range between 62 – 78 units/mg GAE. C. glauca, C. argentea, C. sativum, M. koenigii, Z. officinale, S. nigrum and P. edulis showed moderate level of free radical scavenging (IC_{50} of 4.2 – 8.2 µg GAE/mL) and reducing power (34 – 59 units/mg GAE) activity. The remaining sources such as A. sessilis, A. sylvaticus, A. racemosus, B. alba, C. fenestratum and T. foenum-greacum showed poor antioxidant activities with IC_{50} values between 8.2 to 22.8 µg GAE/mL for DPPH-free radical scavenging and reducing power activity of 27 – 36 units/mg GAE (Table 3 & 4).

Table 3: Phenol content and antioxidant potencies

Plants	Phenolics (mg GAE/g)	DPPH assay IC_{50} (µg GAE/mL)	Reducing power assay (U/mg GAE)
Alpinia galanga	6.51 ± 1.0	2.07 ± 0.2	69.89 ± 6.4
Alternanthera sessilis	6.07 ± 0.5	10.76 ± 0.9	26.96 ± 3.0
Amorphophallus sylvaticus	1.97 ± 0.2	9.34 ± 7.8	36.20 ± 3.0
Asparagus racemosus	4.80 ± 0.5	13.63 ± 1.0	29.22 ± 2.4
Basella alba (leaf)	6.57 ± 0.6	22.85 ± 1.9	27.94 ± 2.2
Basella alba (stem)	2.61 ± 0.1	12.61 ± 1.4	40.63 ± 3.8
Cassine glauca	44.0 ± 3.5	4.45 ± 0.3	56.73 ± 5.9
Celosia argentea	14.67 ± 0.9	5.36 ± 0.4	50.08 ± 4.3
Cocculus hirsutus	31.83 ± 3.1	2.75 ± 0.3	65.17 ± 4.8
Coleus aromaticus	8.45 ± 1.0	3.07 ± 0.3	67.90 ± 7.4
Coriandrum sativum	18.25 ± 2.0	7.14 ± 0.8	43.38 ± 3.9
Coscinium fenestratum	3.34 ± 0.4	12.82 ± 0.9	32.79 ± 3.0
Decalepis hamiltonii	34.43 ± 4.0	0.95 ± 0.1	62.70 ± 4.9
Murraya koenigii	32.27 ± 4.1	5.73 ± 0.4	34.50 ± 2.8
Nigella sativa	12.39 ± 2.0	2.35 ± 0.1	78.08 ± 8.7
Passiflora edulis	4.27 ± 0.5	8.27 ± 0.7	59.01 ± 5.4
Solanum nigrum	18.08 ± 1.6	4.23 ± 0.2	53.26 ± 4.9
Trigonella foenum-greacum	11.55 ± 1.3	10.96 ± 1.4	27.14 ± 2.2
Zingiber officinale	8.89 ± 7.5	6.70 ± 0.5	36.73 ± 4.1

* GAE – gallic acid equivalents.

Discussion

As per our previous studies, compounds/sources exhibiting anti-*H. pylori*, antioxidant and H^+, K^+ -ATPase inhibitory properties were known to possess antiulcer potential *in vivo* [21, 30-31]. During the present investigation this observation was explored further to index antiulcer potential of commonly used dietary sources. The study has an impact on revealing the potential sources that can be employed for gastric health. Antiulcer index (AUI) was calculated based on all the four *in vitro* assays carried out during the study

and categorized the sources as either very good, good, moderate or poor sources.

Phytochemicals, especially phenolics of commonly used dietary sources such as fruits and vegetables are suggested to be the major bioactive compounds for health benefits. Recently plant food components particularly pectic polysaccharides are being implicated with several health benefits including potential gastro protective properties [7, 22, 32-34]. Current study therefore focused on the determination of antiulcer potentials in boiling water extracts of selected dietary/plant sources, which contained generally phenolics as well as polysaccharides.

Results of the study indicated that extracts of different plant sources exhibited differential properties. For example, A. recemosus, C. glauca, C. hirsutus, C. aromaticus, C. fenestratum, D. hamiltonii, N. sativa and Z. officinale exhibited potential anti-H. pylori activity with >90% inhibition when compared to that of the control. However some of these sources particularly, C. glauca, C. aromaticus were very poor in exhibiting H^+, K^+ -ATPase inhibitory properties which is about 4 to 20 folds lesser than other potent anti-H. pylori sources (Table 2). Contrarily, sources like A. sylvaticus, B. alba (stem), S. nigrum, P. edulis and A. galanga which showed potent H^+, K^+ -ATPase inhibition with IC_{50} of less than 30 µg GAE/mL, exhibited poorer anti-H. pylori activity (Table 2) indicating that differential structure may be required for effective intervention in killing H. pylori or modulating stress/ulcerogen induced upregulation of H^+, K^+ -ATPase level during ulcer pathogenicity. Similarly, some of the sources which showed higher anti-H. pylori activity such as A. recemosus, T. foenum-greacum etc., exhibited poorer antioxidant property (Table 2 & 3). Therefore it is difficult to select the potent sources and is important to arrive at the total antiulcer potential. In the current study, the relative percent activity in each assay was calculated and average from all the four assays was considered and depicted as antiulcer Index (AUI). Based on this, sources were categorized as very good (>80 – 100%), good (60-80%), moderate (30-60%) and poor (<30%) sources as depicted in Table 4. Accordingly D. hamiltonii showed the highest (81%) antiulcer index followed by N. sativa (68%).

Our earlier studies had indicated that there is a reciprocal relationship between antioxidant and H^+, K^+ -ATPase or *H. pylori* inhibitory properties [31]. In the current study we could still dissect out further and could reveal that sources which are good antioxidants still may show better anti-*H. pylori* activity although they differ in their potency.

Table 4: Grading of plants for total antiulcer activity based on antiulcer index.

Plants	% Relative activity				Antiulcer Index	Grade
	H. pylori inhibition	ATPase inhibition	DPPH assay	Reducing power		
Decalepis hamiltonii	98.8	44.91	100	80.3	81.0	Very good
Nigella sativa	98.73	35.25	40.42	100	68.6	Good
Coscinium fenestratum	99.58	100	7.41	41.99	62.2	Good
Zingiber officinale	95.08	90.72	14.17	47.04	61.7	Good
Alpinia galanga	60.65	38.2	45.89	89.51	58.5	Moderate
Cocculus hirsutus	96.49	18.99	34.54	83.46	58.3	Moderate
Coleus aromaticus	94.94	6.4	30.94	86.96	54.8	Moderate
Passiflora edulis	66.8	58.81	11.48	75.57	53.1	Moderate
Cassine glauca	100	4.71	21.34	72.65	49.6	Moderate
Trigonella foenum-greacum	69.34	70.96	8.66	34.75	45.9	Moderate
Asparagus racemosus	94.79	29.37	6.97	37.42	42.1	Moderate
Amorphophallus sylvaticus	27.42	83.13	10.17	46.36	41.7	Moderate
Solanum nigrum	23.62	51.2	22.45	68.21	41.3	Moderate
Murraya koenigii	71.02	14.91	16.57	44.18	36.6	Poor
Coriandrum sativum	34.6	31.27	13.3	55.55	33.6	Poor
Celosia argentea	18.35	14.75	17.72	64.13	28.7	Poor
Basella alba - Leaf	24.75	45.48	4.15	35.78	27.5	Poor
Basella alba - Stem	19.09	29.32	7.53	52.03	26.9	Poor
Alternanthera sessilis	25.31	14.88	8.82	34.52	20.8	Poor

Conclusion

In conclusion, the study provided the method to select the potent antiulcer source having multi-potent antiulcer activity based on antiulcer index (AUI). The study revealed Decalepis hamiltonii as 'very good' (AUI of 81) followed by Nigella sativa, Coscinium fenestratum and Zingiber officinale as 'good' (AUI of 61 - 68), and remaining sources as 'moderate' (AUI of 41 to 59) and 'poor' (AUI of 20 to 37) sources. Thus the results of the current study contribute towards validation of the traditional use of selected plant sources for gastrointestinal disorders.

Authors' contributions

BMS carried out the research work and drafted the manuscript. SMD participated in its design and coordination and helped to draft the manuscript.

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