Anti-*Helicobacter pylori* and anti-gastric cancer activity of *Syzygium alternifolium* fruits

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ABSTRACT

Syzygium alternifolium (SA) is a plant widely used in folk medicine. The current study was carried out to characterize the anti-*Helicobacter pylori* (Hp) and anti-gastric cancer activity of *S. alternifolium* fruit extracts. *S. alternifolium* total phenolics (SATP) and *S. alternifolium* aqueous extract (SAAE) were used to examine anti-*H. pylori* and *H. pylori* urease inhibitory activity. The anti-gastric cancer activity of these extracts was also evaluated using an MTT cell viability assay followed by cell cycle progression analysis. The *in vitro* results showed that both extracts have dose-dependent anti-*H. pylori* and anti-gastric cancer activity. SATP has significant anti-*H. pylori* activity against the Hp26695 and HpP12 strains with MIC_{50} values of 148±20 and 141±30 μg/ml, respectively, while SAAE has moderate activity compared to the antibiotic amoxicillin. SATP showed significant urease activity with an IC_{50} of 98±6.4 μg/ml, while SAAE showed a moderate effect compared with positive control. Moreover, SATP showed significant inhibition of human gastric adenocarcinoma cell growth by inhibiting the G2/M phase of the cell cycle with an IC_{50} of 77.9±0.2 μg/ml, while SAAE exhibited moderate inhibition compared with normal control. Reverse phase UV-HPLC analysis indicated the presence of the phenolic compounds cinnamic, gallic, ferulic and vanillic acid in these extracts. The results suggest that SATP and SAAE extracted from *S. alternifolium* have significant anti-*H. pylori* and anti-gastric cancer activity and offer protection against gastritis. Hence, these extracts are recommended for patients with gastrointestinal cancer.

Keywords

Helicobacter pylori

Urease

Gastric cancer

Cell viability

Cell cycle

Introduction

Helicobacter pylori is a gram-negative, spiral-shaped, micro-aerophilic bacterium and major aetiological agent causing chronic gastroduodenal infections (gastritis, peptic ulcers and gastric cancer) [1, 2]. *H. pylori* infections have a prevalence of about 40% in developed countries and 80–90% in developing countries [3]. The rate of colonization of *H. pylori* varies by geographic location and economic status [4]. *H. pylori* typically infects the individual in early life and can persist for months, years or decades without inducing any obvious clinical symptoms [5]. The outcome of infection is believed to be affected by several factors: an excessive or inappropriate immune reaction of the host, bacterial polymorphisms, and environmental factors including lifestyle, hygiene and diet [6].

H. pylori releases the enzyme urease that converts urea to ammonia, which counters stomach acid and creates a neutralizing environment to protect the bacterium from the acids secreted in the stomach [7], which contributes to the pathogenesis of *H. pylori*-induced peptic ulcers [8]. Hence, inhibition of urease activity has been proposed as a possible strategy to eradicate *H. pylori* colonization [9]. Urease (urea amidohydrolase, EC 3.5.1.5) is the largest heteropolymeric enzyme, and consists of UreA (Mr 30,000) and UreB (Mr 66,000) subunits, while the active site contains two Ni^{2+} ions [10]. Active site amino acids of *H. pylori* urease were principally conserved in all known ureases. Hence, the catalytic mechanism of the enzyme is believed to be same [11].

Although the gastric mucosa is well protected from other bacterial infections, *H. pylori* survives by developing pro-

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tective adaptive mechanisms, and persistently colonizes the stomach by evading the immune response. The maintenance of *H. pylori*-mediated chronic inflammation and its regulatory mechanisms are not well understood [12]. Complete eradication of *H. pylori* and treatment of gastrointestinal disorders usually involves the administration of combined treatment with two or more antibiotics (ie, amoxicillin, clarithromycin, metronidazole) and proton-pump inhibitors (ie, lansoprazole or omeprazole).

A persistent gastric ulcer can cause gastric cancer and other complications if not treated promptly. Gastric ulcers are generally not cancerous in the first 3–5 years, but the risk increases with time [13]. Moreover, triple therapy for the eradication of *H. pylori* following gastric ulceration is not always successful. The organism gradually acquires resistance to antibiotics, which reduces treatment efficacy. Unfortunately, the drugs used to treat gastric ulcers, gastric cancer and *H. pylori* infections are complex and have significant adverse effects, resulting in relapse since complete cure is not always achieved. Hence, there is an urgent need to identify new sources of drugs derived from plants to combat *H. pylori* infection.

Our previous studies have reported that three flavonoids, namely 5-hydroxy-7,4′-dimethoxy-6,8-di-C-methylflavone, kaempferol-3-O-β-D-glucopyranoside and kaempferol-3-O-α-L-rhamnopyranoside from the fruit extract of *Syzygium alternifolium* show anti-gastric cancer and anti-*H. pylori* activity [14, 15], while the two compounds quercetin and pinitol from *Indigofera barberi* have antibacterial activity against multidrug-resistant gram-negative bacterial infections [16]. Earlier studies have described many natural plant-derived extracts and compounds which possess anti-ulcer, anti-*H. pylori* and anti-gastric cancer activity. Except for few reports on *Syzygium* species, no systematic investigations have been carried out on *S. alternifolium*, an endemic plant found in the Tirumala hills, in the Seshachalam area of Tirupati, AP, India, with particular reference to its anti-*H. pylori* and anti-gastric cancer activity.

In view of the importance of natural medicinal plants for the eradication of *H. pylori* and anti-gastric cancer activity, the present study investigated the anti-*H. pylori* and anti-gastric cancer activity of extracts derived from *S. alternifolium*.

Materials and methods

Chemicals and reagents

Folin-Ciocalteu reagent, amoxicillin, bovine serum albumin, fetal calf serum, fetal bovine serum, horse serum, vancomy-cin, nystatin, trimethoprim, MTT (3-(4,5–dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), Hams F-12K media, propidium iodide and gallic, cinnamic, ferulic and vallinic acids used as phenolic acid standards, were purchased from Sigma Aldrich, HiMedia and Merck.

Plant material and extraction

S. alternifolium fruits were collected from the Seshachalam hills of Tirumala, Tirupati, and the plant was identified by Dr. K. Madhav Chetty, Department of Botany, Sri Venkateswara University. A voucher sample under accession no. SA135 has been deposited in the herbarium of the university. The collected plant material was air dried, ground to a particle size of 20 mesh and preserved in dry conditions at 4°C until further extraction.

A 10 g aliquot of defatted powder was mixed with 50 ml of ethanol:water (80:20, vol/vol) and left to stand at room temperature for 48 hours. The sample was collected in screw-cap tubes and the suspension was subjected to ultrasonication at a 60% duty cycle for 1 hour at 4°C, followed by centrifugation at 7,500 rpm for 15 min. The clear supernatant was subjected to charcoal treatment to remove pigments. The residue was re-extracted twice and the pooled supernatant was evaporated under vacuum centrifuge. The concentrated dried sample was stored at 4°C for further analysis and referred to as *S. alternifolium* total phenolics (SATP).

Next, 10 g of defatted powder was mixed with 100 ml of sterile double-distilled water, boiled for 5 min, cooled and centrifuged at 5,000×g for 10 min. The clear supernatant was separated and concentrated at room temperature using a vacuum centrifuge. The concentrated fraction was stored at 4°C and referred to as *S. alternifolium* aqueous extract (SAAE).

Determination of total phenolic content

The total phenolic content of SATP and SAAE was determined using Folin-Ciocalteu (FC) reagent assay [17]. A 900 µl sample of water, 1 ml of diluted FC (1:2, v/v) and 2 ml of 10% sodium carbonate solution were added to 100 µl of the test sample. Absorbance was measured at 765 nm with a UV-visible spectrophotometer after incubation for 2 hours at room temperature. Gallic acid was used as the standard for the calibration curve. The total phenolic content was expressed as mg of gallic acid equivalents (GAE) per gram sample. The assay was performed in triplicate.

Culture of *H. pylori*

H. pylori strains Hp26695 and HpP12 were used for in vitro

studies and routinely cultured either on GC agar plates containing 10% horse serum, supplemented with vancomycin (10 µg/ml), nystatin (2 µg/ml) and trimethoprim (2.5 µg/ml), or Brucella Broth containing 10% fetal calf serum under a microaerophilic environment (10% CO_2, 85% N_2, 5% O_2 and >95% relative humidity) at 37°C for 48 hours [18].

Broth microdilution method

H. pylori susceptibility testing was performed using the broth microdilution method. SATP and SAAE were dissolved in 10% DMSO and further diluted with sterile double-distilled water to a concentration of 50–1,000 µg/ml. The samples were suspended in 100 µl of Brucella Broth supplemented with 5% fetal bovine serum in 96-well microtitre plates. *H. pylori* grown for 48 hours in liquid culture was diluted with Brucella Broth and 100 µl of this was incubated into each well to give a final concentration of 1×10^6 CFU/well. The plates were incubated for 48 hours in a microaerophilic atmosphere at 37°C. Amoxicillin was used as positive control and 0.9% saline as negative control. After the incubation period, *H. pylori* growth was assayed by measuring absorbance at 625 nm.

Extraction of *H. pylori* urease

For the urease inhibition assay, 50 ml of *H. pylori* broth culture containing 10^6 cells/ml was centrifuged (5,000×g, 4°C) for 5 min to collect bacteria and the removed pellet was re-suspended in 30 mM Tris-HCl (pH 8.1). The resultant suspension was re-centrifuged (10,000×g) at 4°C for 10 min. The pellet was vortexed with 20% sucrose in Tris-HCl and cells were re-suspended in phosphate buffer at pH 7 and incubated on ice with 30 mg/l protease inhibitor for 30 min. These cells were then ruptured by sonication for 60 s. Soluble and insoluble fractions were separated by centrifugation for 15 min (15,000×g, 4°C). The obtained supernatant was used as the source of *H. pylori* urease.

Urease inhibition assay

The reaction mixture, comprising 25 µl of *H. pylori* urease solution, 55 µl of phosphate buffer solution (3 mM, 4.5 pH) and 100 mM urea, was incubated with 10 µl of plant extract (ie, SATP and SAAE in concentrations ranging from 25 to 500 µg/ml) for 15 min at 30°C in 96-well microtitre plates. The amount of ammonia produced was used to determine urease activity using the indophenol method [19]. After incubation, 40 µl of phenol reagent containing a mixture of 1% phenol, 0.005% sodium nitroprusside and an appropriate amount of alkali (NaOH) reagent, was added to each well.

Increasing absorbance at 630 nm was measured using a microplate reader after incubation for 50 min at 37°C. The percentage of inhibition was calculated using the formula 100–(OD test well/OD control)×100. Acetohydroxamic acid (AHA) was used as the standard inhibitor.

Cytotoxicity assay using the MTT method

AGS (human gastric adenocarcinoma) cells, procured from the National Centre for Cell Sciences, Pune, were grown as a monolayer culture in Hams F-12K medium supplemented with 10% fetal calf serum and 1% penicillin–streptomycin. The culture was incubated in a humidified atmosphere containing 5% CO_2 and 95% air at 37°C. For evaluation of cytotoxicity, the cells were seeded in 96-well microtitre plates at ~10^4 cells per well, and cultured at 37°C for 24 hours. The plant extracts (SATP and SAAE) were added at concentrations of 50–1,000 µg/ml and further incubated for 48 hours. At the end of incubation, MTT reagent was dissolved in DMSO and added into each well at a concentration of 0.2 mg/ml, followed by incubation at 37°C for 4 hours in the dark [20]. The culture medium containing MTT was aspirated off and dye crystals were dissolved in 100 µl of DMSO. The viable cells were detected by reading the absorbance of formazan at 570 nm using a microplate reader. The results are expressed as the 50% inhibitory concentration (IC_{50}), the dose capable of killing 50% of the cells compared to control.

Cell cycle analysis by flow cytometry

AGS (7×10^5) were plated in 6-well cell culture plates, then treated with the plant extracts SATP and SAAE at concentrations of 200, 400 and 800 µg/ml and incubated for 48 hours in a CO_2 incubator. After incubation, the cells were harvested and suspended in PBS and incubated with RNase (20 µg/ml) for 30 min. The cells were then incubated with propidium iodide (50 µg/ml) for 30 min in the dark. The cell cycle distribution was then determined using a FACSAria flow cytometer (FACSDiva v. 6.1.3, BD Biosciences). The percentage of cells in each treated group was calculated as the number of total cells in the treated group/number of total cells in the negative control group×100.

HPLC-UV analysis of SATP and SAAE

The phenolic compounds in the SATP and SAAE extracts were characterized by reverse phase-high performance liquid chromatography (RP-HPLC) (Model LC20A, Shimadzu, Japan) on a C_{18} analytical column. The HPLC system is equipped with two solvent delivery systems, a UV/Vis detector and a Rheodyne injection valve with a 20 µl filling

loop. Resolution of peaks was accomplished by solvent A (phosphoric acid:water 1:99, vol/vol) and solvent B (methanol) at a flow rate of 1 ml/min. Qualitative analysis of individual components was monitored at 220 nm. The standard phenolic acids cinnamic, gallic, ferulic, vanillic and caffeic, were used as reference to identify phenolic compounds in the sample extract [21].

FT-IR spectral analysis

The functional groups of the phenolic compounds in the SATP and SAAE extracts were subjected to FT-IR spectral analysis. The samples were recorded on a Bruker Alpha-Eco FTIR spectrometer (Bruker, Germany) with a reflection sampling module equipped with a ZnSe liquid transmission cell.

Molecular docking studies

Analysis of docking to the active site region of *H. pylori* urease was performed with the phenolic compounds identified in SATP and SAAE (through HPLC analysis) in order to validate inhibition of the enzyme by SATP and SAAE. The docking process was carried out using AutoDock Vina software [22] which recognizes the generated grid of binding pockets in the target protein, and utilizes the ligand and protein PDBQT files as input files, while the grid parameter file (GPF) and docking parameter file (DPF) are generated as output files [23].

Results

Estimation of total phenolic content

The total phenolic contents in SATP and SAAE were estimated by Folin-Ciocalteu reagent assay using gallic acid as standard. The results showed that the total phenolic contents in SATP and SAAE were 18.2±1.2 and 7.8±2.3 mg GAE/g, respectively, with percent yields of 1.82 and 0.78 g/100 g.

Broth microdilution assay

The anti-bacterial activity against two clinical strains of *H. pylori*, Hp26695 and HpP12, was assayed by the broth microdilution method. The minimum inhibitory concentration (MIC_{50}) levels of SATP and SAAE were determined for these clinical isolates. SATP and SAAE showed significant anti-*H. pylori* activity against the Hp26695 strain with MIC_{50} values of 146.06±20 and 182.95±29.7 µg/ml, respectively. Similarly, SATP and SAAE exerted significant bactericidal activity against HpP12 with MIC_{50} values of 141.6±30.9 and 200.03±18.6 µg/ml, respectively, compared with amoxicillin which had an MIC_{50} value of 18.2–23.4 µg/ml.

In vitro cytotoxicity evaluation

We evaluated the cytotoxicity of the plant extracts SATP and SAAE at concentrations of 50–1,000 µg/ml against a gastric cancer AGS cell line using the MTT assay. SATP showed significant growth inhibition with an IC_{50} of 77.9±10.2 µg/ml, while SAAE showed moderate growth inhibition with an IC_{50} of 178.6±15.2 µg/ml. The assay results demonstrate that the extracts induce massive cell rounding, shrinkage and separation from the surface of the culture plates and also indicate that SATP had higher activity and induced apoptotic cell death (Fig. 1a and b).

Cell cycle progression assay

To examine the impact of these plant extracts on cell proliferation, the effect of different concentrations (200, 400 and 800 µg/ml) of SATP and SAAE on cell cycle progression was studied using flow cytometry analysis. The results clearly demonstrate that both the extracts significantly arrest the G2/M phase. Cells treated with SATP and SAAE at 800 µg/ml showed significant reductions in cell count in the G2/M phase, with cell counts of 13.63±0.66% and 12.46±1.97%, respectively (Fig. 1c and d). The data suggest that SATP and SAAE significantly inhibit the G2/M and S phases of AGS cell growth.

Urease inhibition assays

Urease enzyme is an essential virulence factor secreted by *H. pylori* which produces ammonia so that it can survive in the acidic environment of the gastric mucosal layer, as ammonia neutralizes gastric acids. In the present study, the SATP and SAAE extracts at concentrations of 20–200 µg/ml were evaluated for urease inhibition with AHA used as the positive control. The results demonstrated that SATP significantly inhibits urease with an IC_{50} of 98.6±6.4 µg/ml, with SAAE showing moderate urease inhibition with an IC_{50} of 154.1±10.2 µg/ml, when compared to the positive control AHA, which had an IC_{50} of 58±4.2 µg/ml (Fig. 2a).

HPLC fractionation analysis of total phenolics

The HPLC-UV chromatogram of SATP and SAAE revealed that anti-*H. pylori, H. pylori* urease and H+/K+ ATPase inhibitory activity and gastric protective activity were higher in SATP than SAAE. Therefore, the various phenolic compounds in the SATP and SAAE extracts were screened. The HPLC-UV chromatograms of SATP and SAAE showed five peaks at 220 nm in both extracts at the retention times of 4.82, 8.8, 27.3 and 38.4, indicating cinnamic, gallic, ferulic and vanillic acids, and one unknown peak at the retention time of 7.2, and

were compared with the retention times of standard phenolic compounds (Fig. 2b). The concentrations of these phenolic compounds in SATP and SAAE are reported in Table 1.

FT-IR spectral analysis

The four important fractions identified by RP-HPLC in SATP and SAAE were also investigated using infrared spectral characterization. The main functional groups present in the identified fractions of the extracts were carboxylic, methoxy,

hydroxyl and conjugated carboxylic acids. The main functional group carboxylic acid is characterized by the appearance of bands at 1,606 and 1,616 cm^{-1} which specifically represent a carboxylic group. The broad bands at 3,266 and 3,253 cm^{-1} indicate the presence of a hydroxyl group in the fractions. The hydroxyl group represents both the carboxyl group and the phenolic group, as evidenced by the presence of a very high, intense and broad band. This also supports bonding by possible electrostatic interactions of hydroxyl
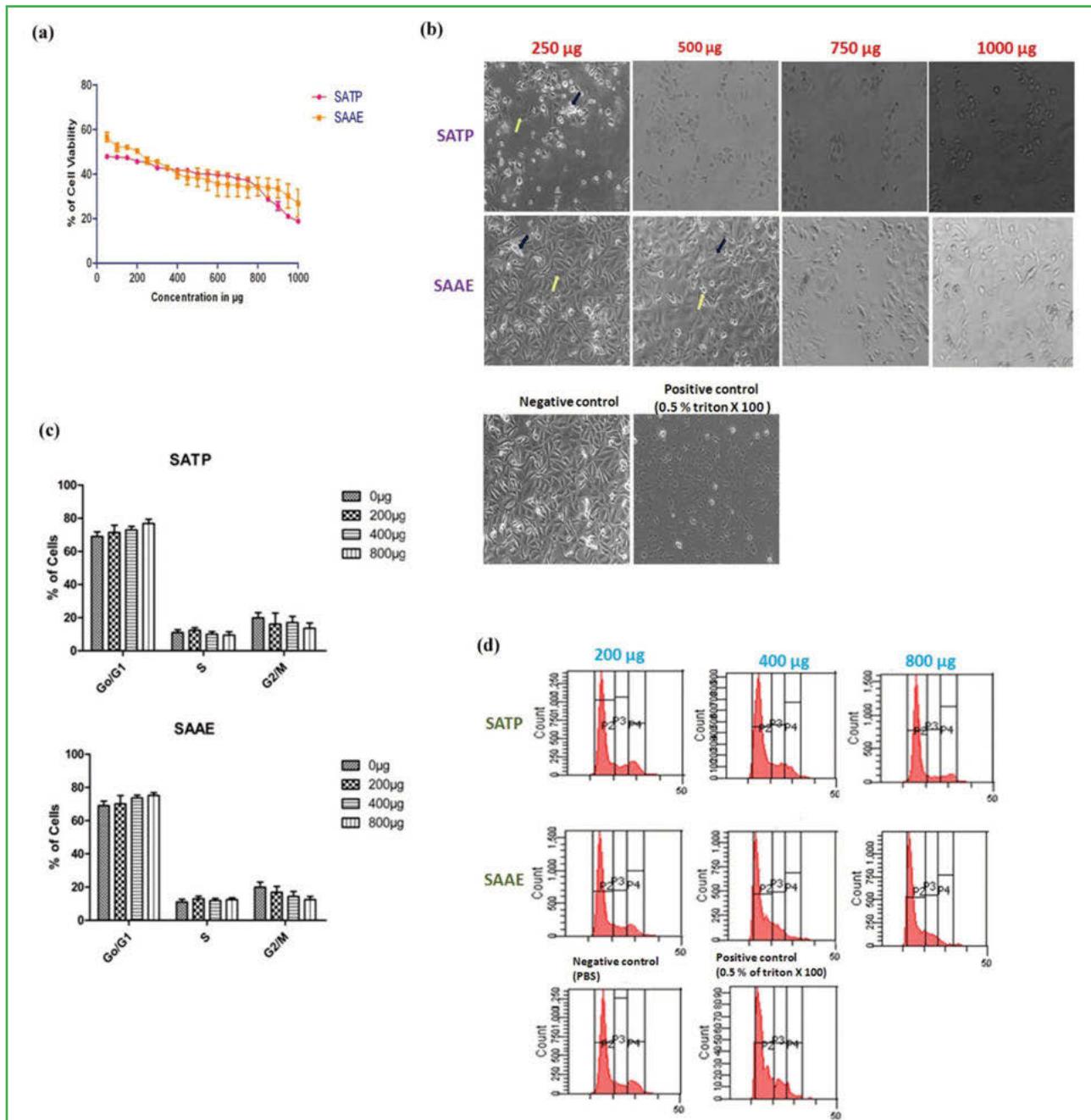
Figure 1 - (a) Cytotoxicity of SATP and SAAE in human gastric cancer cells (AGS). Cells were exposed to extracts at concentrations of 50–1,000 µg/ml. (b) Induction of AGS cell death by SATP and SAAE, after 48 hours of incubation with SATP or SAAE in 250, 500, 750 and 1,000 µg/ml concentrations at 37°C in an atmosphere of 5% CO$_2$. The morphological changes in AGS cells were observed under light microscopy. (c, d) Effect of SATP and SAAE on cell cycle progression of harvested gastric cancer cells (AGS) which were stained with PI and subjected to flow cytometric analysis. All assays were done in triplicate and values are the mean±SD of three replicates in each group SAAE *Syzygium alternifolium* aqueous extract; SATP *S. alternifolium* total phenolics

Sample	Phenolic compound	Retention time	Phenolic (mg/g)	
			SAAE	SATP
1	Cinnamic acid	4.8	17.3±5.586	162.4±19.90[a]
2	Gallic acid	8.8	4.9±1.628	124.73±12.34[b]
3	Ferulic acid	27.3	0.86±0.305	10.8±1.4[c]
4	Vanillic acid	38.4	1.9±0.264	13.93±3.74[d]

Values are means±SD of three replicates. Values with different superscript letters (a–d) are significantly different from SAAE at $p < 0.05$, according to one-way ANOVA coupled with Duncan's multiple-comparison test (SPSS v. 16.0).
SAAE Syzygium alternifolium aqueous extract; *SATP S. alternifolium* total phenolics

Table 1 - The relative concentrations and retention time of phenolic compounds identified in SAAE and SATP

groups with other functional groups containing electronegative centres in inter-molecular or intra-molecular regions. This supports the presence of –OH and –O–CH$_3$ in the same molecule, representing vanillic acid. The bands observed at 2,935 cm^{-1} correspond to the aromatic C–H stretch, confirming the hydroxyl group in the phenolic form. The presence of bands at 1,028 and 1,029 confirms the presence of ether C=C–O–CH$_3$ linkage corresponding to the methoxy functional group which occurs in ferulic acid (Fig. 3).
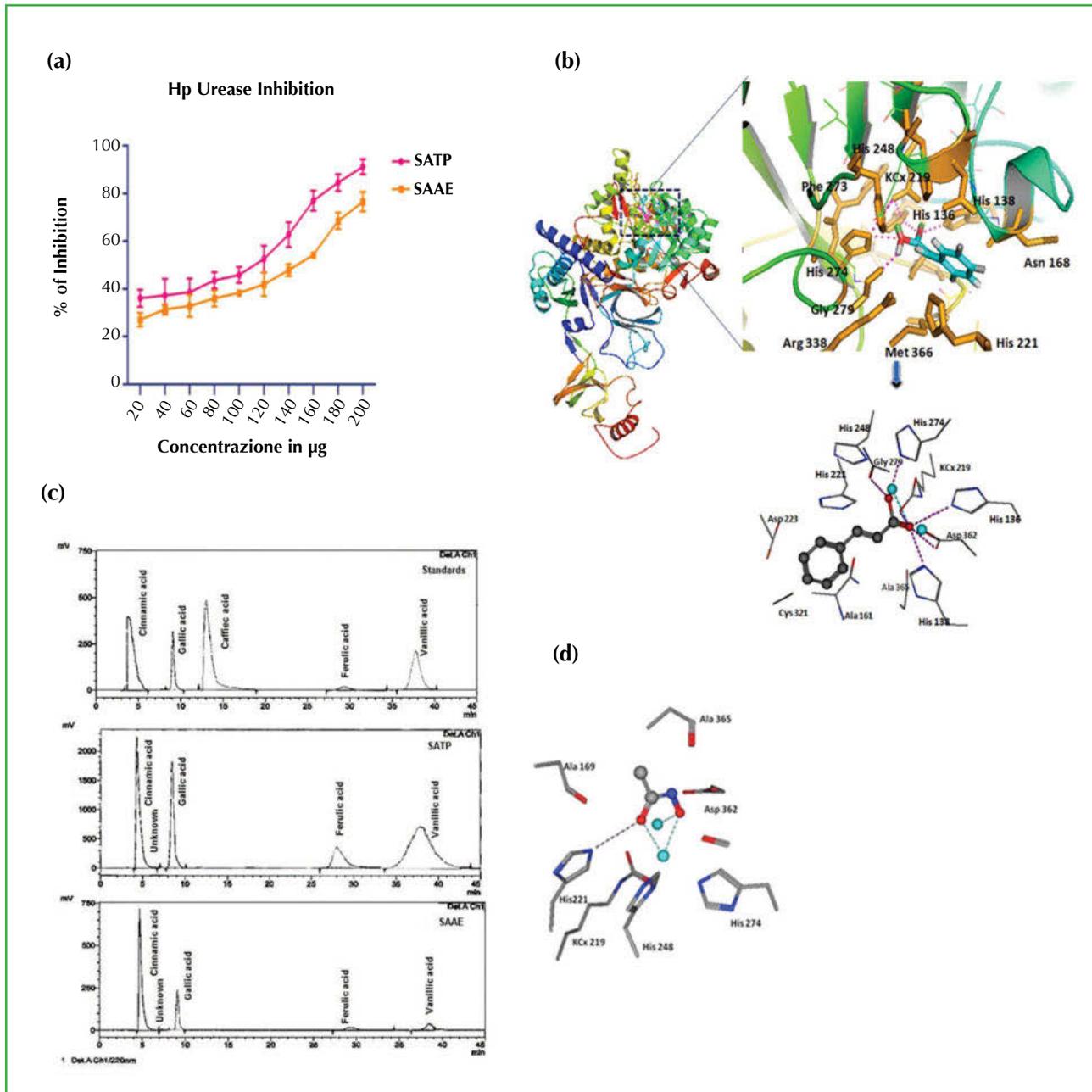
Figure 2 - **(a)** Inhibition of *Helicobacter pylori* urease by SATP and SAAE in concentrations of 20–200 µg/ml. **(b)** RP-HPLC-UV chromatogram of phenolic compounds of SATP and SAAE at 220 nm and compared with known standards. **(c)** Binding mode and molecular interaction of cinnamic acid with the active site of *H. pylori* urease. **(d)** Interaction of urease inhibitor, acetohydroxamic acid (AHA). The residues interacting with cinnamic acid are shown in orange and Ni^{2+} ions are represented by cyan-coloured balls. SAAE *Syzygium alternifolium* aqueous extract; SATP *S. alternifolium* total phenolics
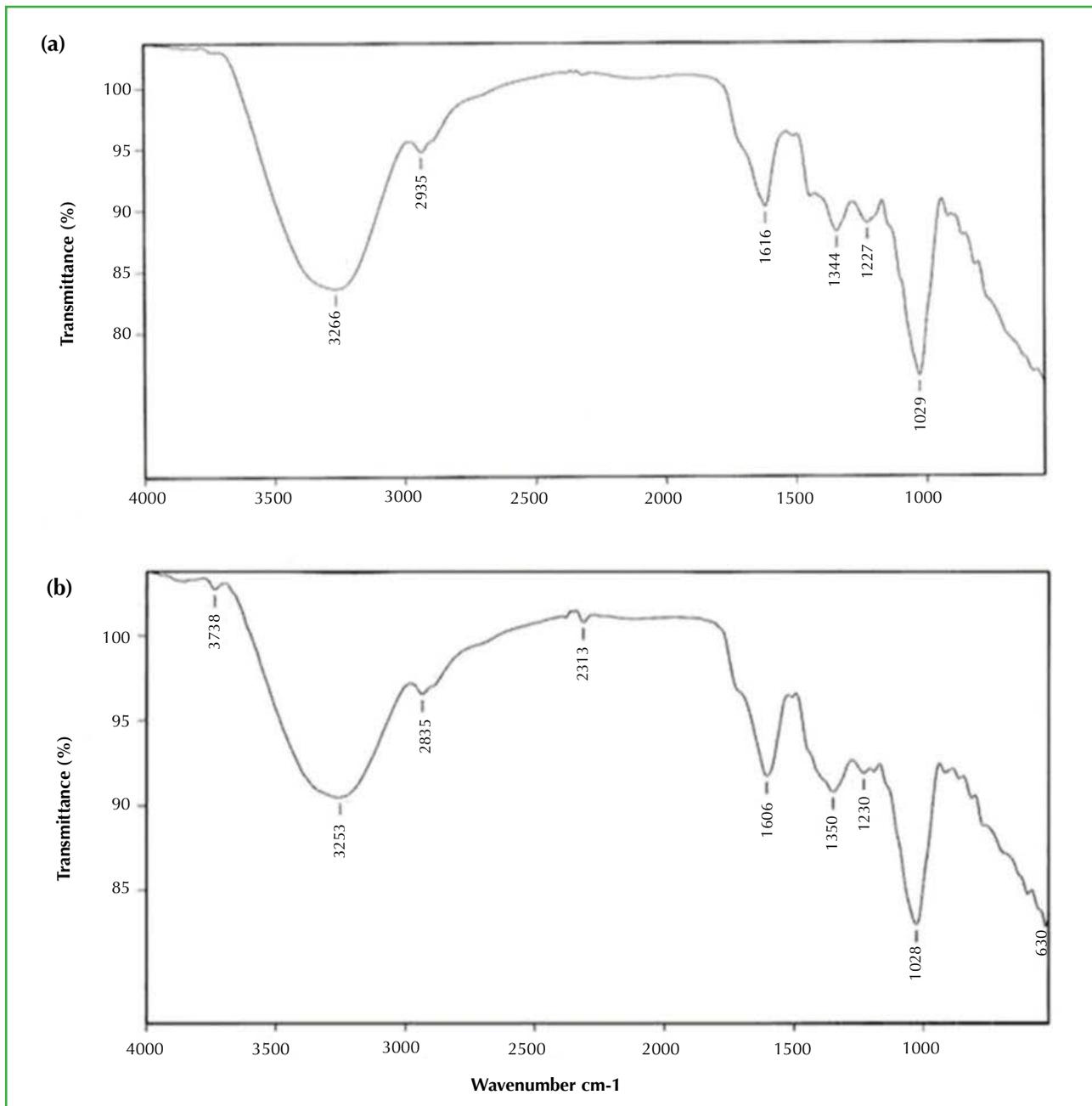
Figure 3 - FT-IR spectral reports of (a) *S. alternifolium* total phenolics (SATP) and **(b)** *S. alternifolium* aqueous extract (SAAE)

Molecular docking analysis

In light of the marked inhibitory activity of SATP and SAAE against *H. pylori* urease, docking analysis was performed for identified phenolic compounds in SATP and SAAE with the inhibitor binding site of *H. pylori* urease. The crystal structure of *H. pylori* urease (PDB ID: 1E9Y) was retrieved from the RCSB Protein Data Bank, which is co-crystallized with its inhibitor AHA. In the binding site, an oxygen atom of the OH group of AHA forms two ionic bonds with two Ni^{2+} atoms; an oxygen atom of the keto group forms one ionic bond with one Ni^{2+} atom and one hydrogen bond with the NH group of His221 (Fig. 2d).

In the co-crystal structure of urease, AHA was removed from the active site, and the energy of the structure was optimized by energy minimization using the CHARMM27 force field. The *H. pylori* urease structure was subjected to docking with identified phenolics in SATP and SAAE into the prepared grid box of the inhibitor binding site. The results showed that all the phenolic compounds (ie, cinnamic, ferulic, vanillic and gallic acids) have significant binding energies of −11.7, −10.8, −10.4 and −9.7 kcal/mol, respectively, towards the inhibitor binding site of *H. pylori* urease (Table 2).

Cinnamic acid has highest binding affinity of −11.7 kcal/ mol and its C=O group formed four hydrogen bonds with

Sample	Name	Binding energy	Interaction		Bond type	Bond length (Å)
1	Cinnamic acid	−11.7	His 136 NE	O	H-don	2.97
			His 138 NE	O	H-don	2.66
			KCX 219 OQ	O	H-don	2.48
			His 274 NE	O	H-don	2.68
			Gly 279 O	H	H-don	1.96
			Asp 362 OD	O	H-don	2.50
			Ni	O	Ionic	2.45
2	Ferulic acid	−10.8	His 136 NE	O	H-don	2.93
			His 138 NE	O	H-don	2.69
			KCX 219 OQ	O	H-don	2.49
			His 274 NE	O	H-don	2.64
			Gly 279 O	H	H-don	1.90
			Asp 362 OD	O	H-don	2.49
			Ni	O	Ionic	2.43
3	Vanillic acid	−10.4	His 136 NE	O	H-don	2.96
			His 138 NE	O	H-don	2.67
			KCX 219 OQ	O	H-don	2.52
			His 274 NE	O	H-don	2.60
			Gly 279 O	H	H-don	1.86
			Asp 362 OD	O	H-don	2.47
			Ni	O	Ionic	2.48
4	Gallic acid	−9.7	His 138 NE	O	H-don	2.49
			KCX 219 OQ	O	H-don	2.48
			KCX 219 OQ	H	H-don	1.14
			Asp 362 OD	O	H-don	2.49
			Ni	O	Ionic	2.60
			Ni	O	Ionic	2.70
5	Acetohydroxamic acid (AHA) positive control	−11.5	His221NE	O	H-acc	2.95
			Ni	O	Ionic	2.48
			Ni	O	Ionic	2.39
			Ni	O	Ionic	2.57

SAAE Syzygium alternifolium aqueous extract; *SATP S. alternifolium* total phenolics

Table 2 - Bonding characterization and binding energy of phenolic compounds identified in SATP and SAAE against *Helicobacter pylori* urease

pounds from natural medicinal plants. In this study, we investigated the ability of SATP and SAAE to inhibit the growth of two clinical isolates of the Hp26695 and HpP12 strains. The results indicate that SATP has significant anti-*H. pylori* activity against Hp26695 and HpP12.

In addition, we have extracted urease from *H. pylori* culture and examined the urease inhibitory activity of SATP and SAAE using the indophenol method. The results show that the extracts exert significant urease inhibition compared with the positive control (AHA). Urease is an essential enzyme which allows *H. pylori* to survive in very acidic conditions in the gastric mucosa of the stomach lumen. Hence, the plant extract may reduce the proliferation of *H. pylori* by inhibiting urease activity and might also inhibit some other molecular targets (ie, Cag A and Vac A) involved in bacterial pathogenesis to eradicate *H. pylori* growth. SATP and SAAE extracts were characterized by RP-HPLC and FT-IR spectral analysis, which revealed the presence of four simple phenolic acids (ie, cinnamic, gallic, ferulic and vanillic acids) in both extracts. The anti-*H. pylori*, anti-urease activity of the extracts may be due to the presence of these phenolic acids which possess antimicrobial and antioxidant activity and offer protection against gastrointestinal disorders.

In order to confirm the in vitro urease inhibition by SATP and SAAE, computational molecular docking of the identified phenolic compounds into the inhibitor binding site of *H. pylori* urease was performed. The results show that the phenolic compounds have significant binding affinity towards *H. pylori* urease in competition with the known *H. pylori* urease inhibitor, AHA. Cinnamic and ferulic acids showed strong affinity and formed six hydrogen bonds with the binding site residues and one ionic bond with an Ni^{2+} ion, which acts as a cofactor for urease activity. Both in vitro

the four amino acids His136, His138, KCx219 and Asp362, and one ionic bond with one of the Ni^{2+} ions. The OH group formed two hydrogen bonds with the amino acids His274 and Gly279 (Fig. 2c).

Discussion

During the past few decades there has been global interest in traditional, complementary and alternative medicine. Many earlier research studies have reported that numerous medicinal plants exhibit potent gastroprotective activity in the ethanol/HCl-induced ulcerated rat model and demonstrate anti-*H. pylori* activity. In the present study we show that *S. alternifolium* total phenolics (SATP) and *S. alternifolium* aqueous extract (SAAE) have anti-ulcerogenic properties against ethanol/HCl-induced ulceration.

H. pylori causes asymptomatic chronic infection when it colonizes the stomach and duodenum, which can lead to peptic ulcers and gastric cancer. Due to the growing antibiotic resistance of the organism to available antibiotics, efforts are being made to identify novel bioactive com-

and in silico studies on *H. pylori* urease inhibition reveal that SATP and SAAE have good inhibitory activity against *H. pylori* growth and the urease enzyme due to the presence of the phenolic compounds, cinnamic, gallic, ferulic and vanillic acid.

Ethanol damages the gastric mucosa and continuous and prolonged exposure to alcohol causes unregulated proliferation of gastric epithelial cells, leading to extensive metaplasia and possibly gastric cancer. Gastric cancer is the second most common cause of cancer-related death [24]. In our study, we found that SATP and SAAE inhibited the growth of a gastric cancer AGS cell line in a dose-dependent manner. The characterized phenolic compounds present in the plant extract may inhibit AGS cell growth by arresting the G2/M and S phases of the cell cycle and inducing apoptotic cell death.

Conclusions

In conclusion, SATP and SAAE have significant anti-*H. pylori* and anti-gastric cancer activity against AGS cells. In light of these results, we believe that these extracts may be an effective treatment for *H. pylori* infection and gastric cancers.

Conflict of interest
The authors declare that they have no conflict of interests.

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