East African Medical Journal Vol. 96 No. 3 March 2019
ANTIMICROBIAL ACTIVITY OF *ALOE SECUNDIFLORA* AGAINST CLINICAL ISOLATES OF *HELICOBACTER PYLORI*
Stephen Njoroge, Department of Medical Microbiology, Jomo Kenyatta University of Agriculture & Technology, P.O Box 62000-00200, Nairobi, Kenya, dsznjoroge@gmail.com,Catherine Mwangi, Department of Medical Microbiology, Jomo Kenyatta University of Agriculture & Technology, P. O. Box 62000-00200, Nairobi, Kenya, Kimang'a Nyerere, Department of Medical Microbiology, Jomo Kenyatta University of Agriculture & Technology, P. O. Box 62000-00200, Nairobi, Kenya, Fredrick Odhiambo, Department of Monitoring and Evaluation, National Public Health Laboratory, P. O. Box 20750-00200, Nairobi, Kenya, Justin Tirimba, Department of Biomedical Science, Technical University of Kenya, P. O. Box 52428, Nairobi, Kenya, Gunturu Revathi, Department of Pathology, Aga Khan Hospital University, P. O. Box 37002-00100, Nairobi, Kenya.

Corresponding author: Stephen Njoroge, Department of Medical Microbiology, Jomo Kenyatta University of Agriculture & Technology, P.O Box 62000-00200, Nairobi, Kenya, dsznjoroge@gmail.com

ANTIMICROBIAL ACTIVITY OF *ALOE SECUNDIFLORA* AGAINST CLINICAL ISOLATES OF *HELICOBACTER PYLORI*

S. Njoroge, C. Mwangi, K. Nyerere, F. Odhiambo, J. Tirimba and G. Revathi

ABSTRACT

Objective: **To evaluate the antimicrobial activity of methanol and aqueous crude extracts of *Aloe secundiflora (A. secundiflora)* against clinical isolates of *Helicobacter pylori* which is the most prevalent cause of gastrointestinal infections.**
Methods: **The agar diffusion method was used to determine the susceptibility of 23 clinical isolates of *H. pylori* to the methanol and aqueous crude extracts of *A. secundiflora*. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was determined by micro well dilution method. The presence of secondary metabolites was determined. The potential bioactive compounds were identified by Gas Chromatography-Mass Spectrometry (GC-MS).**
Results: **Both methanol and aqueous crude extracts of *A. secundiflora* demonstrated antimicrobial activity with highest mean zone diameter of 28 ± 0.47 mm for methanol extracts and 11±0.81mm for aqueous extracts. Lowest recorded MIC and MBC was between 0.19-0.39mg/ml for methanol and aqueous extracts respectively. There was no statistically significance difference (p>0.05) in potency of the extracts in different isolates of *H. pylori* tested both in MIC and MBC. Phytochemical screening of methanol and aqueous crude extracts of *A. secundiflora* showed presence of secondary metabolites such as alkaloids,**

saponin, tannins, flavonoids, and steroids. A total of 8 bioactive compounds were identified GC-MS analysis.
Conclusion: **The present study provides evidence that methanol and aqueous extracts of *A. secundiflora* possess inhibitory prospects against clinical isolates of *H. pylori.***

INTRODUCTION

Helicobacter pylori is a gram negative spiral bacillus and microaerophilic that colonize human stomach (1). *H. pylori* infection is strongly associated with chronic gastritis, which can trigger gastroduodenal disease and gastric malignancies in man with infection reported to be more than 80% of duodenal and more 60% gastric ulcers (2). *H. pylori* Worldwide, gastric cancer accounts for >720,000 deaths annually (3). It is the commonest digestive cancer, and the second commonest cause of cancer-related mortalities and expenditure (4).
National Institute of Clinical Excellence recommends the following regimen for *H. pylori* eradication: Omeprazole, amoxicillin and clarithromycin (i.e. OAC) for 10 days; bismuth subsalicylate, metronidazole and tetracycline (i.e. BMT) for 14 days; and lansoprazole, amoxicillin and clarithromycin (i.e. LAC) for 10-14 days. High resistance to metronidazole and clarithromycin has been noted on a wide scale, with resistance to tetracycline and amoxicillin also noted but still low in many countries (5).
Over 80% population in Africa use medicinal plants for treatment of various ailments (6) More than 20 % of world plants have been tested for their pharmacological activity against pathogenic bacteria with significant efficacy (7).
Aloe secundiflora plant belongs to the family Liliaceae. This plant is succulent with elongated, pointed leaves. The term Aloe is derived from an Arabic word 'alloeh' meaning 'bitter' which refers to the taste of the liquid contained in the leaves (8). Among its therapeutic properties include antimicrobial activity, Aloe has been used in traditional medicine for treatment of various ailments such abdominal discomforts, burns and dermatitis (9).
Despite its use in traditional medicine to treat gastrointestinal infections by the herbalist there is no scientific report on *A. secundiflora* activity against *H. pylori*. This study therefore aimed at determining the presence of phytochemical antimicrobial compounds and evaluating *in-vitro* antimicrobial activity of crude extract of *A. secundiflora*.

MATERIALS AND METHODS

Collection of A. secundiflora leaves: Mature fresh leaves of *A. secundiflora* were collected in Kitui, Mwingi district-Kenya for the study. The plant was authenticated in the Department of Botany of the National Museum of Kenya in Nairobi and a Voucher specimen (number Pf/21076) deposited at the East African Herbarium at the National Museums of Kenya.

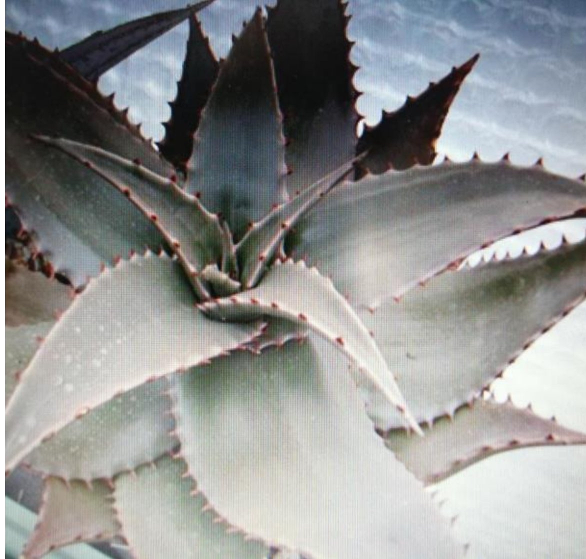

Figure 1. *Aloe secundiflora* plant.

Procedure to obtain crude plant extract: The fresh leaves (Figure 1) were thoroughly washed and dried under a shade at room temperature for 14 days. This ensured they were completely dry without disintegration of bioactive compounds. The dried leaves were then ground to fine powder using laboratory Waring Blender (Clarkson LBC15'S). To prepare organic extracts, 1.5kg of powder was soaked in methanol using an Erlenmeyer flask. Aqueous extracts were also prepared by same amount of powder and soaked in sterile distilled water. The soaked powders were allowed to stand at room temperature for 72 hours, after which they were filtered using Whatman No.1 paper to obtain extracts. To obtain fine powder from the filtered solvent-powder mixture, the methanol extracts were further concentrated in a Rota vapor and aqueous extracts were freeze dried. The resulting fine powders weighed and then transferred in well labeled vials accordingly, as methanol or aqueous extracts.

Procedure to obtain H. pylori isolates: The clinical *H. pylori* were obtained from Gastroenterology Department of Kenyatta National Hospital, Nairobi-Kenya from biopsy tissues of patients with dyspepsia or/and gastrointestinal pain requiring gastroendoscopic examination. The biopsies were obtained from both corpus and the antrum of patient's stomach. Biopsy tissues would be transported to the microbiology laboratory for culture within one hour of collection in 2ml Brain Heart infusion (BHI) broth transport medium. Biopsy tissues were macerated in a sterile mortar with the aid of a sterile fine glass rod to form a homogenate, which was inoculated on Helicobacter-selective agar (Nissui, Tokyo, Japan). The inoculated culture media were incubated at 37 °C (CampyGen gas pack) for 72h under microaerophilic conditions. The isolated bacteria were initially identified as *H. pylori* by colonial morphology, Gram reaction, oxidase reaction, catalase reaction and urease reaction. Confirmation was made by sequencing 16S rRNA gene of *H. pylori.* The isolates were sub-cultured on Brucella agar (BD Company, Cockeysville, MD, USA) supplemented with 5% sheep blood and incubated at 37°C (CampyGen gas pack) for 72h under microaerophilic conditions. A reference strain of *H. pylori* (ATCC 43504) was also included as a positive control. Twenty-three clinical isolates of *H. pylori*, each from a differrent patient were used to assess the *in-vitro* anti-*H. pylori* activity of *A. secudiflora* leaf extracts.

Antimicrobial activity of A. secudiflora leaf extracts:

Agar Well Diffusion

For preliminary determination of antimicrobial activity of crude extracts of *A. secunidflora,* the susceptibility screening of the *H. pylori* to the methanol and aqueous extracts was determined using Agar Well diffusion technique. The Mueller Hinton Blood agar supplemented with sheep blood 5% (MHBA) (OXOID Ltd, Basingstoke Hampshire, UK)

were carefully swabbed with *H. pylori* broth suspension McFarland turbidity standard 3. The inoculated plates were allowed to dry at room temperature for 3-5 minutes. Wells were punched using a sterile cork borer with 6mm diameter. Using a micropipette four wells were filled with 100ml of *Aloe secundiflora* crude extracts 50 mg/mL. One well was filled with 100ml of Ciprofloxacin 2.5 µl/mL as positive control while 10% Dimethyl sulfoxide (DMSO) was use as negative control for methanol Extract and distilled water for aqueous extract. The plates were incubated at 37^{0}C for 72h under microaerophilic conditions after which the diameters of zones of inhibition were measured in millimeters. The experiment was repeated thrice and means zones were recorded. A *H. pylori* control strain, ATCC 43504 inoculated plate was included in all the experiments.

Determination of Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) was determined using the agar dilution method. The MIC was assessed on plant extracts that showed antimicrobial activity by producing inhibition zones (≥ 11mm) during the agar well diffusion assay. Two-fold serial dilution method of the methanol and aqueous extracts *Aloe secundiflora* extract were prepared (0.019mg/ml to 10mg/ml). Each extract was prepared in the test wells in Brain Heart Infusion (BHI) broth supplemented with 5% horse serum (BHI-serum). One hundred microliters of inoculums prepared from 72h colonies growth on Brucella agar supplemented with 5% sterile sheep blood at McFarland turbidity standard 3 was added to 100 μL of the extract-containing culture medium. Control wells were prepared with culture medium and bacterial suspension and broth, only respectively. Ciprofloxacin was used as positive control. DMSO was negative control methanol extract and normal saline for aqueous extract. The plates were covered with a sterile plate sealer and incubated at 37°C for 72h under microaerophilic conditions. The plates were then incubated at 37°C for 72h.

Minimum Bactericidal Concentration (MBC)

After incubation 40 µl of 0.2 mg/µl of INT was added in each of the wells and the plates examined after an additional 45 minutes of incubation. Viable *H. pylori* reduced the yellow dye to pink reddish color (conversion of INT to formazan). The lowest concentration at which the color change was apparently invisible as compared to the next dilution was taken as the minimum inhibitory concentration.

Minimum bactericidal concentration (MBC) was determined by taking 50 µl of suspension from plate wells that demonstrated no growth and inoculating on Brucella agar supplemented with 5% sterile sheep blood. The plates were incubated at 37°C for 72h under microaerophilic conditions.

Procedure to identify phytochemical anti-microbial compounds: To determine the presence of phytochemical compounds in the crude extracts, themethanol and aqueous extracts of *A. secundiflora* leaves were subjected to the following phytochemical screening.

Alkaloids: About 200gm of the extracts was stirred with 2ml of 1%HCl on water bath. One milliliter of the filtrate was treated with five drops of Mayer's reagent and another 1ml was equally treated with Dragendorff's reagent. Formation of an orange precipitate indicated preliminary evidence for the presence of alkaloids.

Saponins: Half a gram (0.5 gms) of the plant extracts was shaken in in a test tube, 5 ml of distilled water and the mixture was allowed to stand for 10 minutes. The stable froth of

more than 1.5 cm and persisting for at least 30 min indicated the presence of saponins.

Tannins: About 200mg of the extract was dissolved in 10 ml of distilled water and filtered. Two drops of 2% (w/v) ferric chloride was added to the filtrate. A blue, black precipitate indicated the presence of tannin.

Steroids: Two milliliters of acetic anhydride were added to 200 mg extracts of each sample with 2 ml of 0.1 M H_2SO_4. The colour change from violet to blue or green ring indicated the presence of steroids.

Flavonoids: 200 mg of extracts was dissolved in 10 ml distilled water and then filtered using Whatman filter No.1. 10mg magnesium turnings were added into 1 ml of the filtrate, followed by the addition of 0.05 ml concentrated HCL acid. The presence of pink red color observed within three minutes confirmed the presence of flavonoids.

Gas chromatography-mass spectrometry (GC–MS) analysis to detect presence of phytochemicals: The presence of bioactive compounds in the was analyzed using GC–MS (SHIMADZU QP2010). The GC specifications were as follows: Column oven temperature set at 70°C, injector temperature 200°C, injection mode-Split, Split Ratio-40, Flow control mode Linear velocity, Column flow 1.51 ml/min, Carrier Gas-Helium 99.99% purity. The MS specifications were as follows: Ion source temperature 200°C, interface temperature 24°C, scan range was 40–1000 m/z, event time-0.5 s, solvent cut time was 5 min, start time was 5 min, end time was 35 min, and ionization was EI (−70 ev).

Data collection and analysis: Data on zones of inhibition and MIC and MBC were collected and coded. It was verified and entered into the social sciences software package (SPSS Version 12), from where it was summarized into means and standard deviations. The data was presented in tables, Chi square test was used to analyze categorical variables. A $p< 0.05$ was considered significant.

Ethical consideration: Ethical approval was obtained from the Kenyatta National Hospital /University of Nairobi-Ethics Research Committee (KNH/UON-ERC) clearance number P241/04/2015.

RESULTS

The antimicrobial activity of *A. secundiflora* methanol and aqueous extract was evaluated by agar well diffusion method to determine zones of inhibition while two-fold serial dilution was used to assay Minimum inhibitory concentration (MIC) was against 23 clinical isolates of *H. pylori* isolated from gastric biopsy from patient referred with various gastrointestinal infections. Both methanol and aqueous crude extracts tested demonstrated anti-*H. pylori* activity. Antimicrobial activity zones of inhibition against the 23clinicalisolates of *H. pylori* are summarized in Table 1. An inhibition zone of ≥ 11 mm by the test bacteria was considered susceptible to the tested methanol and aqueous crude extracts and ciprofloxacin antibiotic. The highest mean zone diameter of 28 ± 0.47 mm was recorded for the methanol extracts.

Table 1
Results of agar well diffusion assay to determine antimicrobial activity of crude methanol and aqueous of A. secundiflora against clinical isolates of H. pylori

H. pylori **isolates**	**Mean zone diameter ± SD (mm)**		
Number	**Methanol Extract**	**Aqueous Extract**	**Ciprofloxacin**
1403571	22±0.47	16±0.47	18±0.47
1507420	20±1.63	18±0.94	20±0.47
1141285	18±0.94	11±0.81	18±1.24
1054085	24±1.24	15±1.24	18±0.47
1746525	24±1.63	15±0.94	20±0.81
1308679	22±0.47	18±0.47	18±0.47
1230281	26±1.63	16±0.47	28±1.24
467001	26±1.24	12±1.24	30±1.24
1883015	18±2.05	12±1.63	18±1.24
1990640	22±0.47	14±0.94	32±0.94
1384003	18±0.94	12±1.63	24±1.70
423885	24±0.47	16±0.81	30±1.63
224148	19±0.47	14±0.94	24±0.81
1062502	28±0.47	18±0.47	20±0.47
1991831	18±0.94	15±0.47	26±0.47
1178234	20±0.47	16±1.24	26±0.94
1874768	22±0.47	14±0.81	20±0.47
199443	20±0.47	14±1.24	22±1.24
1993977	18±0.94	16±0.47	18±1.63
711241	18±0.47	12±1.63	18±1.24
1009070	24±0.47	15±0.47	20±1.63
1940663	18±0.47	12±0.47	22± 1.24
803467	16±0.81	16±0.81	38±0.47
ATCC 43504	22±1.63	18±0.81	37±0.47

MIC values of the methanol extracts ranged from 0.19-0.78 mg/ml and those aqueous extracts and ciprofloxacin 0.39-1.56 mg/mL and 0.01- 0.09mg/mL respectively. There was no significant difference between the methanol and aqueous extractsantimicrobial activity (P > 0.05). The MIC value and zone of inhibition of the methanol extracts of *A. secundiflora* show there was antimicrobial activity against *H.pylori*. The observed results further indicate that methanol could be a superior solvent for the extraction of anti-*H. pylori* compounds from *A.secundiflora* (Table 2).

The MBCvalue (0.19-1.56 mg/ml) was similar to MIC value (0.39-1.56mg/ml) formethanol extracts of *A. secundiflora* (Table 2).

Table 2
Results of antimicrobial activity of methanol and aqueous extract of A. secundiflora against clinical isolates of H. pylori

H. pylori isolates	Methanol Extract		Aqueous Extract		Ciprofloxacin	
Number	MIC mg/ml	MBC mg/ml	MIC mg/ml	MBC mg/ml	MIC mg/ml	MIC mg/ml
1403571	0.78	0.78	1.56	1.56	0.02	0.02
1507420	0.39	0.39	1.56	3.12	0.05	0.05
1141285	0.19	0.39	1.56	3.12	0.05	0.05
1054085	0.39	0.39	0.78	1.56	0.02	0.02
1746525	0.78	0.78	0.39	0.39	0.05	0.05
1308679	0.19	0.19	0.78	1.56	0.04	0.05
1230281	0.39	0.78	0.39	0.78	0.02	0.02
467001	0.78	1.56	0.78	0.78	0.05	0.05
1883015	0.19	0.19	0.39	0.39	0.05	0.05
1990640	0.19	0.19	0.78	0.78	0.05	0.05
1384003	0.19	0.39	0.78	1.56	0.09	0.09
423885	0.78	0.78	0.78	0.78	0.09	0.09
224148	0.78	1.56	0.39	0.78	0.16	0.32
1062502	0.78	0.78	1.56	1.56	0.05	0.05
1991831	0.39	0.78	0.39	0.78	0.05	0.05
1178234	0.39	0.39	1.56	3.12	0.05	0.05
1874768	0.39	0.39	1.56	3.12	0.16	0.16
199443	0.19	0.19	0.78	0.78	0.16	0.16
1993977	0.78	0.78	1.56	3.12	0.32	0.32
711241	0.78	1.56	0.78	1.56	0.16	0.16
1009070	0.19	0.19	0.78	1.56	0.32	0.32
1940663	0.78	1.56	0.39	0.78	0.05	0.05
803467	0.19	0.39	0.78	1.56	0.16	0.16
ATCC43504	0.05	0.05	0.09	0.09	0.02	0.02

According to the qualitative phytochemical screening of methanol and aqueous extracts, phytochemicals were more intense in methanol extract (Table 3).

Table 3
Phytochemical screening of methanol and aqueous extract A. secundiflora

Phytochemical	Methanol Extract	Aqueous Extract
Alkaloids	++	++
Saponins	+++	+
Tannins	+	+
Flavonoids	++	+
Steroids	++	+

Key: +Phytochemical intensity

The spectrum of *A. secundiflora* showed eight prominent peaksduring GC-MS analysis. The main bioactive compounds obtained were1, 2 Benzene dicarboxylic acid, bis (2-methylpropyl) ester, Dibutyl Phthalate, and Phenol, 2,4-bis(1,1-dimethylethyl) (Table 4).

Table 4
Compounds obtained during GC–MS analysis of methanol extract of A. secundiflora leaves

Compound Name	Compound classification	Known bioactivity	Chemical formula	Retention Time (minutes)
Hexadecane	Alkane	Antifungal and antimicrobial	$C_{16}H_{34}$	12.3806
Butylated Hydroxytoulene	Phenol	Antimicrobial and antioxidants	$C_{15}H_{24}O$	12.6832
Phenol,2,4-bis(1,1-dimethylethyl)	Phenol	Antimicrobial and anti-inflammatory	$C_{14}H_{22}O$	12.8334
Tridecane	Alkane	Anti-microbial and anti-cancerous	$C_{13}H_{28}$	14.8916
1,2Benzenedicarboxylicacid, bis(2-methylpropyl) ester	Ester	Antimicrobial and anti-cancerous	$C_{16}H_{22}O_4$	16.6906
Dibutyl Phthalate	Ester	Antimicrobial, antimalarial and antifungal	$C_{16}H_{22}O_4$	17.6564
Oxirane -2-butyl-3methyl-cis	Alkane	Anti-microbial and anti-cancerous	$C_7H_{14}O$	6.150
Octadecanoic acid	Ester	Antifungal and antimicrobial	$C_{22}H_{44}O_4$	16.284

DISCUSSION

Previous studies have demonstrated that *A. secundiflora* has been widely used in Kenya as medicinal plant as a wonder to treat several gastrointestinal and respiratory ailments in human as well as animals (10). However, there is no scientific evaluation of its anti-helicobacter activity. The study revealed that *A. secundiflora* leaf extract had anti-microbial activity against *H. pylori* for both aqueous and methanol extracts.

In this present study, methanol and aqueous extracts of *A. secundiflora* were evaluated for antimicrobial activity against clinical isolates of *H. pylori* from gastric biopsies. The crude extract of *A. secundiflora* exhibited significant inhibitory and bactericidal effects against all the clinical isolates of *H. pylori*. Variation in MIC and MBC values for methanol and

aqueous extract suggested a selective antimicrobial susceptibility at different concentration. The results of this study are in agreement with Fabry *et al*. (11) who indicated that MIC values <8mg/mL of crude extracts were significant. The MBC values obtained for the extracts against the clinical isolates of *H. pylori* are higher than MIC, indicating that the extracts are bacteriostatic at lower concentrations and bactericidal at higher concentrations.

The photochemical study results revealed that *A. secundiflora* contains some very important phytochemicals such as alkaloids, saponins, tannins, flavanoids and steroid compounds which could be responsible for its antimicrobial properties.Secondary metabolites such as alkaloids, saponins, tannins, flavanoids and steroids have shown to possess antimicrobial activity against bacterial pathogens (12). Methanol and aqueous extract from *A. secundiflora* contained alkaloids, saponins, tannins, steroids, and flavonoids. The secondary metabolites present in this plant could be accountable to the anti-*helicobacter pylori* activity observed in this study. Alkaloids have shown to possess pharmacological activity providing need to study its structure in development of antimicrobial agent (13). Saponins are detergent like substances that have been reported to possess antimicrobial activity due to their membrane permeabilizing properties thus lysing microorganism (14). Tannins are known as antimicrobial biomolecules, they have shown inhibitory activity against bacterial pathogens (15). Flavonoids were present in significant amount in this study and by their known antimicrobial activity to inhibit the synthesis of the nucleic acids, and therefore cause permeability of the inner bacterial membrane and a dissipation of the membrane potential of Gram negative (16). Steroids have been reported to possess antimicrobial activity. They exert their action by causing leakage of from liposomes (17).

GC-MS was used to evaluate bioactive compounds present in methanol extract. Hexadecane, Butylated Hydroxytoulene, Phenol,2,4-bis(1,1-dimethylethyl),Tridecane 1,2 Benzene Dicarboxylicacid, bis (2-methylpropyl),Dibutyl Phthalate Oxirane -2-butyl-3methyl-cis and Octadecanoic acid have shown to possess antimicrobial and antioxidant activity (18). Butylated Hydroxytoulene (BTH) is phenolic antioxidants with antimicrobial. The antimicrobial activity of butylated hydroxytoluene depends on the presence of a hydroxyl group on the molecule that disrupt the bacterial DNA synthesis and disruption of cellular membrane (19). Currently, *H. pylori* have become multidrug resistant to commonly used antibiotics causing different diseases (20). Because of this, searching of new drugs is important in the recent times and *A. secundiflora* has been proven as a potential source of bioactive molecules.

CONCLUSION

The present study provides evidence that methanol and aqueous extracts of *A. secundiflora* possess inhibitory prospects against clinical isolates of *H. pylori.* The results of this study also complement the ethnobotanical use of *A. secundiflora* in treatment of *H. pylori* infections there could be explored as source of new anti-*helicobacter pylori* agent.

REFERENCE

1. Kalali, B., Formichella, L., & Gerhard, M. (2015). Diagnosis of Helicobacter pylori: Changes towards the Future. *Diseases*, **3** (3), 122–135

2. Amieva, M. R., & El-Omar, E. M. (2008). Host-Bacterial Interactions in Helicobacter pylori Infection. *Gastroenterology*, **134** (1), 306–323.

3. Lee, J. Y., & Kim, N. (2015). *Diagnosis of Helicobacter pylori by invasive test : histology*. **3** (1), 1–8.

4. Uotani, T., & Graham, D. Y. (2015). *Diagnosis of Helicobacter pylori using the rapid urease test*. **3** (1), 1–7.

5. Vega, A. E., Cortiñas, T. I., Puig, O. N., & Silva, H. J. (2010). Molecular characterization and susceptibility testing of Helicobacter pylori strains isolated in Western Argentina. *International Journal of Infectious Diseases*, **14** (SUPPL. 3).

6. Mostafa, A. A., Al-Askar, A. A., Almaary, K. S., Dawoud, T. M., Sholkamy, E. N., & Bakri, M. M. (2018). Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi Journal of Biological Sciences*, **25** (2), 361–366.

7. Mongalo, N. I. (2013). Antimicrobialactivities of selected medicinal plants used to treat sexually transmitted infections in Blouberg area, Limpopo Province. *MSc Dissertation, University of Zululand, KwaDlangezwa, February*.

8. M Msoffe, P. L. (2009). Short Communication The Efficacy Of Crude Extract of *Aloe Secundiflora* on *Candida Albicans*. In *J. Trad. CAM* (Vol. **6**, Issue 4).

9. Hagir OM., et al. “Antimicrobial activity and minimum inhibi- tory concentration of Aloe vera sap and leaves using different extracts”. Journal of Pharmacognosy and Phytochemistry 6.**3** (2017): 298-303.

10. Bjorå, C. S., Wabuyele, E., Grace, O. M., Nordal, I., & Newton, L. E. (2015). The uses of Kenyan aloes: An analysis of implications for names, distribution and conservation. *Journal of Ethnobiology and Ethnomedicine*, *11*(1). https://doi.org/10.1186/s13002-015-0060-0

11. W. Fabry, P. O. Okemo, and R. Ansorg,(1998) “Antimicrobialactivity of East African medicinal plants,” Journal ofEthnopharmacology. **60** (1) 79–84,.

12. Osbourn, A. E., & Lanzotti, V. (2009). Plant-derived natural products: Synthesis, function, and application. In *Plant-derived Natural Products: Synthesis, Function, and Application* (Issue June 2009). 1-597.

13. Cushnie, T. P. T., Cushnie, B., & Lamb, A. J. (2014). Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. *International Journal of Antimicrobial Agents*, **44** (5), 377–386.

14. Arabski, M., Węgierek-Ciuk, A., Czerwonka, G., Lankoff, A., & Kaca, W. (2012). Effects of saponins against clinical E. coli strains and eukaryotic cell line. *Journal of Biomedicine and Biotechnology*, *2012*.

15. Kurhekar, J. (2016). TANNINS – antimicrobial CHEMICAL COMPONENTS. *International Journal of Technology and Science, 5-9*, **IX**(January 2016), 5–9.

16.Cushnie, T. P. T., & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents*, **26** (5), 343–356.

17. Kumaravel, S., & Alagusundaram, K. (2014). Antimicrobial activity and Phytochemical analysis of selected Indian spices. *Journal of Pure and Applied Microbiology*, *8*(5), 4131–4136.

18. Hess, S.C. (1995). Antimicrobialactivity and Phytochemical analysis of *Vochysiadivergens*(Vochysiaceae). *Journal of Ethnopharmacology*, **47**: 97-10033.

19. Endris, A., Asfaw, N., & Bisrat, D. (2016). Chemical composition, antimicrobial and antioxidant activities of the essential oil of *Lippia javanica* leaves from Ethiopia. *Journal of Essential Oil Research*, **28** (3), 221–226.

20. Boyanova, L.; Mitov, I. Geographic map and evolution of primary Helicobacter pylori resistance to antimicrobial agents. Expert Rev. Anti Infect. Ther. 2010, 8, 59–70.