Available online at www.ijpsdr.com

International Journal of Pharmaceutical Sciences and Drug Research
2017; 9(3): 100-104

Research Article

ISSN: 0975-248X
CODEN (USA): IJPSPP

In vitro Anti-*Helicobacter pylori* Activity and GC-MS Analysis of *Enicostemma axillare* (Lam). Raynal

Babu A, Sheik Noor Mohamed M, Jaikumar K, Anand D, Saravanan P*

Department of Botany, Ramakrishna Mission Vivekananda College (Autonomous), Mylapore, Chennai - 600 004, Tamil Nadu, India

ABSTRACT

Traditional healers possess a rich knowledge on the use of medicinal plants for the treatment of various diseases. *Enicostemma axillare* (Lam). Raynal is one plant, used by healer to treat gastric cancer or ulceration. We assessed the phytochemical components of leaf extract for its antibacterial activity, to add value and provide an evidence-base for their traditional use. The antibacterial potential of the leaf extract was tested against *Helicobacter pylori* using agar well diffusion method. The minimum inhibitory concentration (MIC) of ethanol extract exhibited the (MIC) against *H. pylori* ranged from 200µg/ml - 250µg/ml followed by chloroform extract. Gas chromatography and mass spectrum (GC-MS) analysis confirms the occurrence of different components in the ethanol and chloroform leaf extract of the studied species.

Keywords: *Helicobacter pylori, Enicostemma axillare* (Lam). Raynal, GC-MS, Agar well Diffusion.

DOI: 10.25004/IJPSDR.2017.090301 **Int. J. Pharm. Sci. Drug Res. 2017; 9(3): 100-104**

***Corresponding author**: Dr. P. Saravanan
Address: P.G. & Research Department of Botany, Ramakrishna Mission Vivekananda College (Autonomous), Mylapore, Chennai - 600 004, Tamil Nadu, India
Tel.: +91-9444316365
E-mail ✉: sarviveka@gmail.com

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Received: 20 March, 2017; **Revised:** 03 April, 2017; **Accepted**: 09 April 2017; **Published:** 29 May 2017

INTRODUCTION

Helicobacter pylori, a Gram-negative microaerophilic and spiral shaped bacterium that has been implicated in gastric, peptic ulcer disease and over long exposure potentially to gastric carcinoma. [1-2] The eradication of *H. pylori* has thus been utilized as the primary treatment strategy of these diseases for 3 decades. In the past, standard triple therapy consist of a proton pump inhibitor and two broad spectrum antibiotics (usually Amoxicillin, Clarithomycin, Metronidazole) was able to achieve eradication rates > 90%. [3] In view of the incomplete cure achieved with antibiotics-based therapy due to resistant strains. Investigations on naturally occurring antimicrobial compounds found in dietary, medicinal plants and herb extract as alternative source of antibiotics for treating *H. pylori* infection have excellent potential.

Enicostemma axillare (Lam). Raynal (Gentianaceae), vernacular name (Vellarugu) Indian white head is a perennial herb growing up to 40 cm tall, with 4-angled stems. This species is globally distributed in West Indies, tropical Africa, India and Sri Lanka. Leaves are narrow-oblong, lance shaped. Stalkless white flowers are borne in dense clusters in leaf axils. Plant extracts were reported for the biological activities such as antidiabetic, anti-inflammatory, stimulant, astringent, diuretic and useful in skin disease. [4-5] A number of medicinal preparations in the ayurvedic system of Indian Medicine were recommended for the treatment of gastric ulcers. Though they are claimed to offer significant relief, their usage is in vogue since centuries. However, no effort has been made to identify the phytochemical constituents responsible for the anti helicobacter activity of the plant. A variety of botanical products have been reported to possess antiulcer activity but the documented literature has centered primarily on pharmacological action in experimental animals. Hence an attempt has been made to prove the efficacy of *E. axillare* in the treatment of gastric ulcer and to assess the phytochemical constituents of the plant.

MATERIALS AND METHODS

Collection of plant material

The healthy mature plants of *Enicostemma axillare* were collected during the month of April-May in 2016, from Thuthipattu and Karuvatchi village of Villupuram District, Tamil Nadu. Collected plant was authenticated as *E. axillare* (Lam). Raynal at the Botanical Survey of India, Southern Regional Centre, Tamil Nadu. Herbarium of the plant specimen was deposited in the Department of Botany, Ramakrishna Mission Vivekananda College (Autonomous), Chennai, Tamil Nadu, India.

Extraction of Bioactive compound from plant material

Fresh leaves from healthy plants were shade dried, and pulverized to fine powder using mechanical grinder. A portion of the dried powdered leaves (50 g) was placed in a soxhlet apparatus and the extraction was performed with 750 ml of different solvents like ethanol and chloroform for 8 h separately at a temperature not exceeding the boiling point of the respective solvent. Extract obtained was filtered through a 45µm filter. The resulting solution was concentrated in a vacuum to dryness to give ethanol and chloroform extract. The extract was stored in a refrigerator at 4°C until further use.

GC-MS analysis

The chemical composition of the ethanol and chloroform extract was established by GC-MS analysis. The analysis was performed on a Clarus 680 GC-MS system in Mass Spectrometer clarus 600 (EI), Software Turbo Mass ver 5.4.2. The injector temperature was set at 260°C during the chromatographic run. The 1µl of extract sample injected into a split ratio of 1/10, the instrument the oven temperature was as follows: 60°C (2 min); followed by 300°C at the rate of 10°C min^{-1}; and 300°C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240°C; ion source temperature 240°C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library. The name, molecular weight and structure of the components of the test materials were ascertained.

Bacteria and Cultural Conditions

Clinically isolated strains of *Helicobacter pylori* culture were identified and used for this experiment. *H. pylori* were cultivated on Columbia blood agar (Oxoid, UK) at 37°C for 72 h. Plates were incubated in anaerobic conditions.

Agar Well Diffusion Method

The agar diffusion assay was performed on Columbia blood agar as described earlier. [6] The test organism (*H. pylori*) was swabbed over the medium using sterile cotton buds. Aliquots of 85µl of each test-sample solution of different concentrations (100µg, 150µg, 200µg and 250µg) were applied into 10 mm diameter agar wells. After incubation in anaerobic conditions at 37°C for 72 h, the diameter of the clear zone (no growth) around the well in the bacterial lawn was measured and used to express the antimicrobial activity. The inhibition zone diameter was measured in millimeters (mm). The tests were performed in triplicate and the final results were presented as the arithmetic average.

Statistical analysis

The results were expressed as mean ± SD of the triplicates. One way ANOVA was applicable and used to analyze level of statistical significance. $P<0.05$ were considered statistically significant. [7]

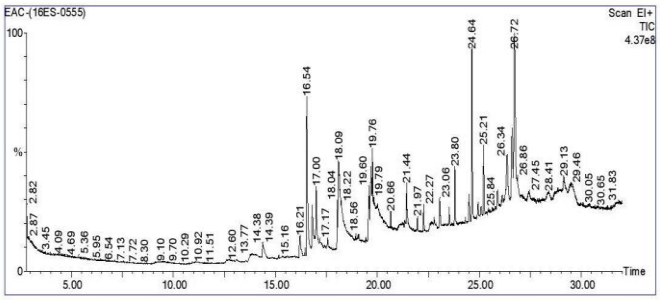


Fig. 1: GC-MS profile of chloroform leaf extract of *Enicostemma axillare* (Lam). Raynal

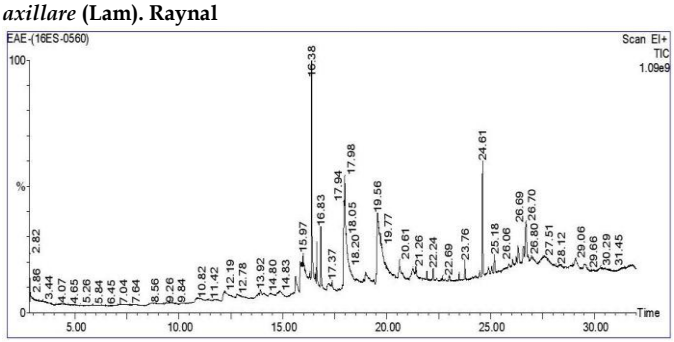


Fig. 2: GC-MS profile of ethanol leaf extract of *Enicostemma axillare* (Lam). Raynal

RESULTS AND DISCUSSION

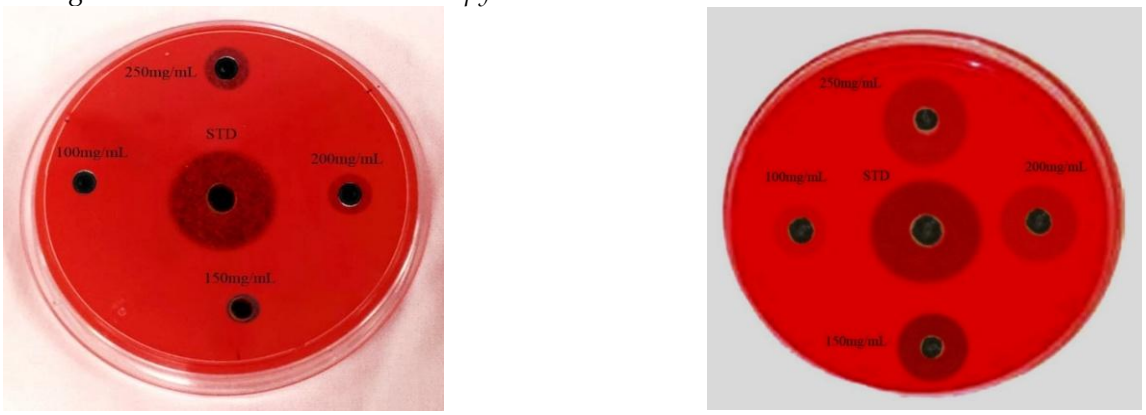
GC-MS analysis

The components present in the chloroform and ethanol leaf extracts of *E. axillare* was identified by GC-MS analysis. The mass spectra of all the phytochemicals identified in both chloroform and ethanol leaf extract of *E. axillare* were presented in Figure (1 and 2). The GC-MS analysis revealed the presence of 12 and 10 compounds in ethanol and chloroform leaf extract respectively (Table 1 and 2). Premiere phytochemical analyses of the methanolic extract of *E. axillare* leaves revealed the presence of alkaloid, flavonoids, tannins, glycosides. [8] The various phytochemicals which contribute to the medicinal activities of the plant were shown in Table 2 are based on Dr. Duke's phytochemical and ethnobotanical Databases. [9] Out of the bioactive components identified, the ethanol leaf extract showed the presence of Erythrocentaurin (RT 15.839) has antimicrobial properties as reported earlier. It was found that Erythrocentaurin was identified in GC-MS analysis of methanolic extract of *E. axillare,* which supported the work. [10] Erythrocentaurin is a metabolite of swertiamarin which is a secoiridoid glycoside.

Anti-ulcer activity of *Enicostemma axillare* (Lam).Raynal against *Helicobacter pylori*

The ethanol and chloroform extract of *E. axillare* inhibited the growth of clinical isolates of *H. pylori* in agar well diffusion method. [21] The results of the anti pylori activity of the investigated extracts are shown in Table 4. Different solvents are used for extraction of antimicrobial compounds form plants, and the success is largely dependent on the type of solvent used. [22] In *E. axillare,* the effect of ethanol extract reported in this study is in line with many others that have documented the good extracting ability of methanol from plants.

It was observed in *E. axillare* that chloroform extract, ethyl acetate and hydro alcoholic extracts showing prominent antimicrobial activity against *Bacillus subilis*, *Staphylococcus aureus*, *E. coli*, *Shigella sonni*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Aspergillus niger*, *Candida albicans* as compared to aqueous and methanol extracts. However our results also indicate that the chloroform extract showed prominent activity in line with ethanol extract at 250µg/ml concentration. Our result, in comparison of chloroform and ethanol observed ethanol to be the best in terms of the diversity of compounds extracted. [23] This antimicrobial activity may be due to the presence of compound 'Erythrocentavrin' in ethanol extract, which is already reported to possess antimicrobial properties. According to duke data base many compounds isolated also possess antimicrobial properties.



A. Chloroform leaf extract of *Enicostemma axillare* (Lam). Raynal **B. Ethanol leaf extract of *Enicostemma axillare* (Lam). Raynal**

Fig. 3: *In vitro* Antiulcer activity of ethanol and chloroform leaf extract of *Enicostemma axillare* (Lam). Raynal

Table 1: GC-MS profile of chloroform leaf extract of *Enicostemma axillare* (Lam). Raynal

S. No	Name of the compound	Retention Time	Peak %	Molecular Weight	Molecular formula
1	TRICHLOROMETHANE	2.823	32.389	118	$CHCl_3$
2	3,7,11,15-TETRAMETHYL-2-HEXADECEN-1-OL	16.544	9.829	296	$C_{20}H_{40}O$
3	HEXADECANOIC ACID, ETHYL ESTER,	18.095	8.507	284	$C_{18}H_{36}O_2$
4	1-HEXYL-2-NITROCYCLOHEXANE	19.600	2.492	213	$C_{12}H_{23}O_2N$
5	9,12,15-OCTADECATRIENOIC ACID, METHYL ESTER, (Z,Z,Z)-,	19.700	5.094	292	$C_{19}H_{32}O_2$
6	9-TRICOSENE, (Z)-	19.760	3.744	322	$C_{23}H_{46}$
7	1-HEXYL-2-NITROCYCLOHEXANE	19.825	8.598	213	$C_{12}H_{23}O_2N$
8	2,6,10,14,18,22-TETRACOSAHEXAENE, 2,6,10,15,19,23-HEXAMETHYL-,(ALL-E)-,	24.637	6.615	410	$C_{30}H_{50}$
9	TRITETRACONTANE	25.207	2.921	604	$C_{43}H_{88}$
10	2H-CYCLOPROPA[A]NAPHTHALEN-2-ONE, 1,1A,4,5,6,7,7A,7B-OCTAHYDRO-1,1,7,7A-TETRAMETHYL-, (1A.ALPHA.,7.	26.368	3.845	218	$C_{15}H_{22}O$
11	HEPTACOSANE, 1-CHLORO	26.603	4.530	414	$C_{27}H_{55}Cl$
12	2,4,4-TRIMETHYL-3-HYDROXYMETHYL-5A-(3-METHYL-BUT-2-ENYL)-CYCLOHEXENE	26.723	11.436	222	$C_{15}H_{26}O$

Table 2: GC-MS profile of ethanol leaf extract of *Enicostemma axillare* (Lam). Raynal

S. No	Name of the compound	Retention Time	Peak%	Molecular Weight	Molecular formula
1	1,3,6-HEPTATRIENE, 2,5,5-TRIMETHYL	15.613	3.198	136	$C_{10}H_{16}$
2	ERYTHROCENTAURIN	15.839	2.988	176	$C_{10}H_8O_3$
3	BICYCLO[4.2.0]OCTA-1,3,5-TRIENE-7-CARBOXYLIC ACID	15.909	2.992	148	$C_9H_8O_2$
4	BENZAMIDE, N-(4-CYANOMETHYLPHENYL)-2-METHYL	15.974	7.030	250	$C_{16}H_{14}ON_2$
5	3,7,11,15-TETRAMETHYL-2-HEXADECEN-1-OL	16.384	12.732	296	$C_{20}H_{40}O$
6	EICOSANOIC ACID	17.979	29.297	312	$C_{20}H_{40}O_2$
7	9,12-OCTADECADIENOYL CHLORIDE, (Z,Z)-	19.560	17.619	298 l	$C_{18}H_{31}OC$
8	1-HEXYL-2-NITROCYCLOHEXANE	19.720	6.743	213	$C_{12}H_{23}O_2N$
9	2,6,10,14,18,22-TETRACOSAHEXAENE, 2,6,10,15,19,23-HEXAMETHYL-, (ALL-E)-	24.607	6.657	410	$C_{30}H_{50}$
10	2R-ACETOXYMETHYL-1,3,3-TRIMETHYL-4T-(3-METHYL-2-BUTEN-1-YL)-1T-CYCLOHEXANOL	26.698	5.050	282	$C_{17}H_{30}O_3$

Table 3: Biological activity of compounds in leaf extract of *Enicostemma axillare* (Lam). Raynal

S. No	Name of the Compound	Compound Nature	Activity *
1	TRICHLOROMETHANE	Chloroform	anti-virus, anti-cancer, anti-mutagenic, anti-allergic and anti-ulcer [11]
2	3,7,11,15-TETRAMETHYL-2-HEXADECEN-1-OL	Terpene alcohol	Anti-inflammatory Fragrance compound, Antimicrobial, Anti-inflammatory [12]
3	HEXADECANOIC ACID, ETHYL ESTER,	Ester Compound	Antioxidant, Flavor, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Hemolytic, 5-Alpha reductase inhibitor [13]
4	1-HEXYL-2-NITROCYCLOHEXANE	Ketone	Antioxidant, antimicrobial, anti-inflammatory [14]
5	9,12,15-OCTADECATRIENOIC ACID, METHYL ESTER, (Z,Z,Z)-,	Fatty acid ester compound	Anti-inflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistaminic, Antiarthritic, Anticoronary, Antieczemic, Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic [12]
6	9-TRICOSENE, (Z)-	Aliphatic hydrocarbon	Antibacterial, antioxidant [15]
8	2,6,10,14,18,22-TETRACOSAHEXAENE, 2,6,10,15,19,23-HEXAMETHYL-,(ALL-E)-,	Triterpene compound	Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant, Chemopreventive, Lipoxygenase-inhibitor, Pesticide [14]
9	TRITETRACONTANE	Alkane hydrocarbon	No activity reported [16]
10	2H-CYCLOPROPA[A]NAPHTHALEN-2-ONE, 1,1A,4,5,6,7,7A,7B-OCTAHYDRO-1,1,7,7A-TETRAMETHYL-, (1A.ALPHA.,7.	-	-
11	HEPTACOSANE, 1-CHLORO	Alkenes	Antioxidant [17]
12	2,4,4-TRIMETHYL-3-HYDROXYMETHYL-5A-(3-METHYL-BUT-2-ENYL)-CYCLOHEXENE	Essential oil component	Antibacterial, Antioxidant [18]
13	1,3,6-HEPTATRIENE, 2,5,5-TRIMETHYL	Hydrocarbons	Antioxidant [18]
14	ERYTHROCENTAURIN	-	Cytotoxicity against human [19]
15	BICYCLO[4.2.0]OCTA-1,3,5-TRIENE-7-CARBOXYLIC ACID	-	-
16	BENZAMIDE, N-(4-CYANOMETHYLPHENYL)-2-METHYL	-	-
17	EICOSANOIC ACID	-	Anticancer activity (Pubcem)
18	9,12-OCTADECADIENOYL CHLORIDE, (Z,Z)-	Linolenic acid	Anti-inflammatory, Insectifuge, Hypocholesterolemic, Cancer preventive, Nematicide, Hepatoprotective, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor [13]
19	2R-ACETOXYMETHYL-1,3,3-TRIMETHYL-4T-(3-METHYL-2-BUTEN-1-YL)-1T-CYCLOHEXANOL	Fragrance industry	antibacterial, anti inflammatory activities [20]

* Source: Dr. Duke's phytochemical and Ethanobotanical Database.

Table 4: Antibacterial activity of *Enicostemma axillare* (Lam). Raynal against *Helicobacter pylori* using agar well diffusion method.

S. No	Concentration µg/ml	Ethanol extract (Zone of inhibition in mm)	Chloroform extract (Zone of inhibition in mm)
1.	250	21.76 ± 0.68	20.33 ± 0.57
2.	200	18.00 ± 1.00	17.33 ± 0.57
3.	150	15.66 ± 1.15	-
4.	100	13.00 ± 1.00	-
5.	Clarithromycin (Control)	30.66 ± 0.57	28.33 ± 1.15

The data plotted represent Mean ± SD of triplicate experiments (n-3)

Earlier it was reported that anti *H. pylori* activity *in vitro* has been estimated with fruit juices using agar well diffusion method seemed to possess marked antibacterial activity, with a maximum growth inhibition diameter of 19 mm whereas *E. axillare* exhibited considerable growth inhibition with diameter ranging above 20 mm. [6] In General, among the investigated extracts, the ethanol fraction exhibited a higher antiulcer activity effect followed by the chloroform extract. The maximum inhibition zone of

21.76 ± 0.68 mm was shown by ethanol extract at a concentration of 250µg/ml. When the concentrations of the extracts were decreased from 250 - 100µg/ml, a slight decrease in inhibition zone was observed in both the solvent case. Despite all the maximum zone of inhibition zone attained at a concentration of 250 mg/ml by plant extract was in near similar to the standard antibiotic Clarithromycin.

Thus the present investigation reveals that the non-polar and polar extracts of *E. axillare* possess significant bactericidal activity which was analyzed by GC-MS showed the presence of phytoconstituents belonging to the Triterpene, Ketone, Aliphatic hydrocarbon, esters, alcohols, *etc.* The presence of such a variety of phytochemicals may be attributed to the medicinal characteristics of the plant. Further Isolation and characterization of the bioactive compounds may provide novel or lead compounds, which could become a template for the synthesis of alternate anti *H. pylori* drugs.

ACKNOWLEDGEMENT

The authors are thankful to the Secretary and the Principal, Ramakrishna Mission Vivekananda College (Autonomous), Mylapore, Chennai, India for providing all facilities. Authors also thank Botanical survey of India (BSI), Coimbatore, Tamil Nadu for identification and authentication of Plants. We thank SAIF VIT, Vellore for contribution of GC-MS analysis and Armats Biotek Training and Research Institute, Gundy, Chennai – 600 032 for anti *H. pylori* studies.

REFERENCES

1. Suerbaum S, Michetti P. *Helicobacter pylori* infection. New England Journal of Medicine. 2002 Oct 10; 347(15):1175-86.
2. Malfertheiner P, Megraud F, O'Morain C, Bazzoli F, El-Omar E, Graham D, Hunt R, Rokkas T, Vakil N, Kuipers EJ. Current concepts in the management of *Helicobacter pylori* infection: the Maastricht III Consensus Report. Gut. 2007 Jun 1; 56(6):772-81.
3. Malfertheiner P, Megraud F, O'morain CA, Atherton J, Axon AT, Bazzoli F, Gensini GF, Gisbert JP, Graham DY, Rokkas T, El-Omar EM. Management of *Helicobacter pylori* infection—the Maastricht IV/Florence consensus report. Gut. 2012 May 1; 61(5):646-64.
4. The Wealth of India—Raw Materials, First Supplement Series, National Institute of Science Communication, CSIR, New Delhi, Vol.3 D-I, 2002, pp. 80.
5. Rawat RS. Plant based traditional knowledge for improved health care delivery system, workshop: Approaches tow, evaluation of medicinal plants prior to clinical trials. Yashada, Pune, 2006, pp. 18-23.
6. Babarikina A, Nikolajeva V, Babarykin D. Anti-Helicobacter activity of certain food plant extracts and juices and their composition in vitro. Food and Nutrition Sciences. 2011; Oct 17(08):868.
7. Sheik Noor Mohamed. M, Jaikumar. K, Anantha Padmanabhan. S, Deventhiran. M, Anand. D, Saravanan. P. Evaluation of *in vitro* immunomodulatory activity of ethanol seed extract of *Sinapis alba* Linn. Indo American Journal of Pharmaceutical Research. 2016; 6(12): 7149-7154.
8. Zaheer Z, Deshpande SD, Paithankar AP, Khan S, Rana ZA. Phytochemical screening of plant of *Enicostemma axillare*. International Journal of Research in Ayurveda & Pharmacy. 2011; 2(1):175-176.
9. Jadhav V, Kalase V, Patil P. GC-MS analysis of bioactive compounds in methanolic extract of *Holigarna grahamii* (wight) Kurz. International Journal of Herbal Medicine. 2014; 2(4):35-9.
10. Jiang J, Bai L, Surtees JA, Gemici Z, Wang MD, Alani E. Detection of high-affinity and sliding clamp modes for MSH2-MSH6 by single-molecule unzipping force analysis. Mol Cell. 2005; 20(5):771-81.
11. Al-Marzoqi AH, Hameed IH, Idan SA. Analysis of bioactive chemical components of two medicinal plants (*Coriandrum sativum* and *Melia azedarach*) leaves using gas chromatography-mass spectrometry (GC-MS). African Journal of Biotechnology. 2015; 14(40):2812-30.
12. Mohan DN, Sivakama SS, Karuppusamy S, Mohan VR, Parthipan B. GC-MS analysis of leaf and stem bark of *Cleidion nitidum* (Muell.–Arg.) Thw. Ex kurz. (Euphorbiaceae). Asian J. Pharm. Clin. Res. 2014; 7(2):41-7.
13. Sermakkani M, Thangapandian V. GC-MS analysis of *Cassia italica* leaf methanol extract. Asian journal of pharmaceutical and clinical research. 2012; 5(2):90-4.
14. Sivakumar V, Gayathri G. GC-MS analysis of bioactive components from ethanol extract of *andrographis paniculata*. World journal of pharmacy and pharmaceutical sciences. 2011; 4(11): 2031-2039.
15. Mihailovi V, Vukovi N, Niforovi N, Soluji S, Mladenovi M, Maškovi P. Studies on the antimicrobial activity and chemical composition of the essential oils and alcoholic extracts of *Gentiana asclepiadea* L. J Med Plant Res. 2011; 5(7):1164-1174.
16. Elangovan M, Dhanarajan MS, Elangovan I. Determination of bioactive compounds from the Petroleum ether leaf extract of *Moringa oleifera* and *Phyllanthus emblica* using GC-MS analysis. World Journal of Pharmaceutical Research. 2015; 4(3):1284-98.
17. Safayhi H, Sailer ER. Anti-inflammatory actions of pentacyclic triterpenes. Planta medica. 1997 Dec; 63(06):487-93.
18. Sen S, Talukdar NC, Khan M. A simple metabolite profiling approach reveals critical biomolecular linkages in fragrant agarwood oil production from *Aquilaria malaccensis*–a traditional agro-based industry in North East India. Current Science. 2015 Jan 10;108(1):63.
19. Geng CA, Huang XY, Ma YB, Zhang XM, Chen JJ. Synthesis of erythrocentaurin derivatives as a new class of hepatitis B virus inhibitors. Bioorg Med Chem Lett. 2015; 25(7):1568-71.
20. Saravanan D, Kasisankar V. Asharani IV. GC-MS analysis of phytocomponents in the leaves of *Actinodaphne madraspatana* Bedd. Int J Res Pharm Sci. 2013; 4:469-73.
21. Bhagure L, Wagh A. Phytochemical and pharmacological review on *Enicostemma axillare* Linn. (Gentianaceae). Journal of Pharmacy Research. 2012; 5(3):1460-1462.
22. Masoko P, Picard J, Eloff JN. The antifungal activity of twenty-four southern African Combretum species (Combretaceae). South African Journal of Botany. 2007 Apr 30; 73(2):173-83.
23. Njume C, Afolayan AJ, Samie A, Ndip RN. In-vitro anti-Helicobacter pylori activity of acetone, ethanol and methanol extracts of the stem bark of *Combretum molle* (Combretaceae). Journal of Medicinal Plants Research. 2011 Jul 18; 5(14):3210-6.

HOW TO CITE THIS ARTICLE: Babu A, Sheik Noor Mohamed M, Jaikumar K, Anand D, Saravanan P. *In vitro* Anti-*Helicobacter pylori* Activity and GC-MS Analysis of *Enicostemma axillare* (Lam). Raynal. Int. J. Pharm. Sci. Drug Res. 2017; 9(3): 100-104. **DOI: 10.25004/IJPSDR.2017.090301**