Pharmacogn J. 2020; 12(6)Suppl: 1494-1503
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Original Article

GC-MS Analysis of Anti-Enterobacterial Dichloromethane Fraction of Mandukaparni (*Hydrocotyle javanica* Thunb.) – A plant from Ayurveda

Manab Mandal[1], Debabrata Misra[1], Narendra Nath Ghosh[1], Sukhendu Mandal[2], Vivekananda Mandal[1]*

ABSTRACT

Background: Mandukaparni (*Hydrocotyle javanica* Thunb.) is a well known medicinal herb used as folklore medcine in many chronic and infectious gastric and other diseases by the people of Estern Himalayan regions**.** However, the therapeutic active principles of this plant remained unknown. **Objective:** The main objective of the study was to characterize anti-enterobacterial dichloromethane fraction of the volatile oils of it by GC-MS. **Materials and Methods:** In the present study, dichloromethane (DCM) fraction (MP-DCMf) of Mandukaparni was collected by phase separation of the methanol extract and tested for anti-enterobacterial potentiality against human pathogenic gastrointestinal and food poisoning bacteria by agar well diffusion assay, viability assay and LDH assay and SEM studies. Characterization of the active MP-DCMf fraction was performed by TLC and GC-MS analysis. **Results:** The MP-DCMf possessed bio-active compounds that have antibacterial potentiality against both the Gram-positive and Gram-negative bacteria. The MIC and MBC values were in the range from 1.56 mg/ml to 0.78 mg/ml and 6.25 to 1.56 mg/ml, respectively. The time kill assay showed that at a dose of 3.12 mg/ml of MP-DCMf was lethal to the *E. coli* MTCC 723 at the 18th hr of treatment. LDH release had moderate positive correlation with the activity index and time of treatment whereas strong negative correlation with CFU count. It caused highest cell disruption in *S. mutans.* The principal compounds were D-carvon (30.949%);1H-Isoindole-1,3(2H)-dione; 2-(2-chlorophenyl) (28.483%);Cyclohexanone; 2-methyl-5-(1-methylethen) trans (10.04%); D-Limnone (9.256%);2,6-Octadien-1-ol, 3,7-dimethyl- acetate (6.684%); p-Cresol (0.551%), and Thymol (0.118%). Pub-chem database search also supports that these compounds have very strong bactericidal activity by membrane damage as evidenced by LDH and SEM studies. **Conclusions:** MP-DCMf contains many potential antibacterial compounds that can be used to combat the gastrointestinal and food poising bacterial pathogens.

Key words: Anti-enterobacterial, Chemical profiling, Mandukaparni, Time kill assay, Volatile oils.

Manab Mandal[1], Debabrata Misra[1], Narendra Nath Ghosh[1], Sukhendu Mandal[2], Vivekananda Mandal[1]*

[1]University of Gour Banga, Malda, INDIA.
[2]University of Calcutta, Calcutta, INDIA.

Correspondence
Vivekananda Mandal
University of Gour Banga, Malda, INDIA.
E-mail: vivek.bot@ugb.ac.in

History
- Submission Date: 18-06-2020;
- Review completed: 01-08-2020;
- Accepted Date: 08-08-2020.

DOI : 10.5530/pj.2020.12.205

Article Available online
http://www.phcogj.com/v12/i6s

INTRODUCTION

Hydrocotyle javanica Thunb. (*H. javanica*) and *Centella asiatica (L.)* Urban. (*C. asiatica)* which are popularly known as Java pennywort and Indian pennywort respectively are commonly known as 'Mandukaparni' in Ayurveda for resemblance in their leaf shape and used for the ailments of indigestion, dysentery, nervousness and blood impurity as mutual substitute in herbal preparations.[1] *C. asiatica* (known as Gotu kola in Hindi) belonging to the Apiaceae family is an important medicinal plant of the Ayurveda for improving the brain function and delaying ageing process since thousands of years back and listed in the historic '*Sushruta Samhita*', an ancient Indian medical text.[2] Fresh Gotu kola juice is taken along with cow's milk in cough and asthma and for rejuvenation, decoction is taken in fever and dysentery and leaf is used as a vegetable also.[3] The Hydrocotyle genus has almost 100 species that grow in tropical and temperate regions worldwide and belongs to the family Araliaceae, previouslyApiaceae. *H. javanica* grows abundantly in Himalaya and southern part of India known as 'Golpatta' in Nepali, and 'Mahagotukola in Hindi.[4] The leaves of the plant are being used to treat several diseases like gastritis, throat shore, eye and ear infection by various ethnic communities like Bhutia, Damai, Kami, Lepcha, Limbu, Rai, Sherpa and Tamang of Darjeeling Hills (27°3'15.88" N and 88°15'28.10" E, elevation 2034 m) of West Bengal, India.[5] It is also used to treat jaundice. Different biomedical aspects of the species like antibacterial activity and physicochemical parameters of methanol extract had been reported.[6] In Kaushikasutra, Mandukaparni is described as an Tikta (Bitter in taste) Aushadhi (Medicinal herb). Other ancient Ayurvedic texts described the medicinal properties of the herb as follows: It has a Kasaya (Astringent) after taste and a cooling effect. The Guna (Main quality) of the herb is Laghu (Light to digest); and its Virya (Potency) is Sheeta (Cold). Its Prabhava (Action) is Nootropic and neuroprotective. The Dhatu (Tissue) effect is through Rasa (Fluid), Rakta (Blood) and Mamsa (Muscle).[7] Gastro-intestinal ailments like diarrhea and typhoid are one of the major public health problems in developing

Cite this article: Mandal M, Misra D, Ghosh NN, Mandal S, Mandal V. GC-MS Analysis of Anti-Enterobacterial Dichloromethane Fraction of Mandukaparni (*Hydrocotyle javanica* Thunb.) – A plant from Ayurveda. Pharmacogn J. 2020;12(6)Suppl:1494-503.

and developed countries. WHO estimates the diarrhea and typhoid fever diseases among infants and young children caused by various enteric pathogens, responsible for approximately 2.2 million and 0.6 million deaths each year. Some important pathogenic bacteria viz. *Escherichia coli*, *Salmonella* spp., *Shigella* sp., *Vibrio cholera* and *V. parahaemolyticus* are responsible for diarrhea causing more than two million deaths every year.[8] In Indian hospitals, one-third of the total pediatric patients admitted are diarrheal and 17% mortality in indoor pediatric patients are diarrhea related.[9] To combat such fatal disease many potent antibiotics have been used for the last few decades like Rifaximin, Metronidazole, Ciprofloxacin but the recurrent and rampant use of certain drugs lead to development of resistance among these pathogens. Antibiotics also have been associated with adverse effects which include hypersensitivity, allergic reactions and immune suppression.[10] Many pathogens like *S. typhi* show multidrug resistance (MDR) against antibiotics.[11] In these circumstances, plants have the major advantages for the cheapest, less side effect and effective alternative sources of drugs. Therefore, the pharmaceutical sector is in a need to develop potent alternative anti-enterobacterial compounds to treat these drug resistant pathogens. Most of the cases, to develop such potential drug, the scaffold of the molecules of natural product is used.[12] The aim of the present study is to characterize the antibacterial constituents of the bioactive DCM fraction of Mandukaparni and to evaluate its mechanism of antibacterial action.

MATERIALS AND METHODS

Plant material

The Mandukaparni plant (*H. javanica*) was collected from Darjeeling hill areas of West Bengal, India. The voucher specimen (vide reference no. DGC/SP-02) was identified and authenticated at Central National Herbarium, Indian Botanic Garden, Shibpur, Howrah, India.

Extraction and fractionation of bioactive components

The bioactive components of the Mandukaparni powder were extracted by Soxhlet extractor using 80% aqueous methanol solvent and concentrated by rotary vacuum evaporator following the methodology of Mandal *et al.*[13] The MP-DCMf fraction from the methanol extract was recovered with 1/4th volume of DCM using orbital shaker for 48 hours. The recovered fraction was evaporated to dryness and used for further study.

Anti-bacterial activity assay

The gastrointestinal pathogenic bacterial strains used in this study were procured from MTCC, Chandigarh, India, and MCC, Pune, India and were cultured in respective culture conditions. Antibacterial activity of the MP-DCMf (25 mg/ml) was evaluated by the agar wells diffusion method.[14] Ciprofloxacin (50 µg/ml) and DCM (100%) were used as positive and negative control, respectively. The potentiality was assessed by activity index by calculating the ratio of diameter of zone inhibition of the extract and diameter of inhibition zone of antibiotic.

Determination of MIC and MBC and time kill assay

MIC was determined using 1.3% (w/v) Mueller Hinton agar (MHA) medium with the conc. range of MP-DCMf (25 mg/ml to 0.75 mg/ ml).[13] MBC and bactericidal kinetic assay were done by applying greater than the MIC dose on the actively grown bacterial strain (log phase growth) of *B. cereus* MTCC 1272, *E. coli* MTCC 723, *E. faecalis* MCC 2041T and *S. mutans* MTCC 497 and the viability was evaluated by plate count method after incubating at 37°C for 96 hrs.[14] Time kill measurement was done at ½, 1, 2, 4, 8, 16 and 20hrs.[15] The culture without treatment was considered as negative control.

Study on mode of antibacterial action: cellular integrity and cell morphology

The effect of MP-DCMf on cellular integrity was evaluated by lactate dehydrogenase (LDH) assay. Sonicated (100% amplitude, at 0.9 cycles for 5 min) [Probe Sonicator, PKS-250F, PCI Analytics, USA] test strain was taken as the positive control. Cell-free supernatant was taken at ½ h, 1 h, 2 h, 8h and 16 h of treatment time and the amount of LDH released in the cell free supernatant was measured using standard procedure. One unit of LDH was calculated as 1 µmol of nicotinamide adenine dinucleotide reduction (NADH) per minute. The effect of MP-DCMf on the cell morphology was confirmed by SEM after treatment at 2 × MIC dose on *L. monocytogenes* MTCC 657 following the protocol of Mandal *et al.*[13]

GC-MS analysis

The active compounds present in the MP-DCMf was analysed by using the GC-MS instrument (Model 7890B GC-240 ION TRAP MS, Agilent Technologies, USA) equipped with a capillary column, VF-5MS (Length-30m, ID-0.25 mm, Film-0.25 µm, Max temp-325 °C) with the mass detection range of m/z 10-1000.The instrument was operated in electron impact mode at an emission current of 25 µAmps, maximum ion time of 65000 µSeconds, target TIC of 20000 counts, with injector temperature at 250 °C, and detected at 300 °C.The identification of compounds was done by matching the mass spectral records of NIST library and the Mole % of the compounds was determined by the formula: Mole % = $A_i/A_c \times 100$, where, A_i = Peak area count of individual compound and A_c = Cumulative peak area count.

Thin layer chromatographic analysis

TLC analysis was done in Silica Gel 60 F_{254} precoated aluminium sheet (Merck, Germany) and developed in n-hexane-ethyl acetate (3:2, v/v) solvent system as mobile phase and detected under UV254 nm. The chemical group of the active fraction was evaluated by comparative TLC with standard flavonoids (Quercetin) and phenols (Gallic acid) using two different solvent systems, viz. chloroform: methanol (96:4, v/v) and toluene: ethyl acetate: methanol: formic acid (3:3:0.8:0.2, v/v), respectively, and detected under UV_{254nm}. For alkaloids, chloroform: ethanol (9:1, v/v) was used as the solvent system and visualized by spraying with Dragendoff's reagent. The R_f values were measured and compared with the standards.

Statistical analysis

Antimicrobial activities were calculated as mean ± SE. The correlation between LDH release and activity index, LDH release and time of treatment, and LDH release and Log CFU count were analyzed by Spearman's 2-tailed bi-variate method and linear regression model using MS – Excel 2007.

RESULTS

Antibacterial activity of the MP-DCMf

The *in-vitro* antibacterial assay of the MP-DCMf demonstrated broad antibacterial activity against both Gram-negative and Gram-positive bacterial strains.The activity index of the bioactive fraction showed the highest value (0.866) against *E. coli* MTCC 723 and the lowest (0.822) in case of *E. faecalis* MCC 2041T. Apart from antibacterial activity against the gastrointestinal pathogens it had also potential activity index (0.675) against the topical pathogen, *S. aureus* MTCC 96, and food toxigenic *B. cereus* as shown in Table 1.

MIC, MBC and bactericidal kinetic of the MP-DCMf

The MICs were in the range from 3.12 mg/ml to 0.78 mg/ml. The MIC value for *B. cereus, E. coli, S. typhimurium* was 0.78 mg/ml while for *B. subtilis, L. monocytogenes, S. mutans,* was 1.56 mg/ml, for the

E. faecalis, S. aureus and *V. parahaemolyticus* it was 3.12 mg/ml. The MBC values were in the range from 6.25 to 1.56 mg/ml. MBC value for *B. cereus, E. coli, S. typhimurium* was 1.56 mg/ml while for *B. subtilis, L. monocytogenes, S. mutans* it was 3.12 mg/ml and for *E. faecalis, S. aureus and V. parahaemolyticus* it was 6.25 mg/ml.The time kill assay showed that at a dose of 3.12 mg/ml, the MP-DCMf was lethal to the *E. coli* MTCC 723 at 18th hrs of treatment (Figure 1) which indicated that the active fraction had a potential bactericidal effect on the treated bacterial strain.

Mode of action of the MP-DCMf

LDH activity and its correlation

The LDH activity of MP-DCMf on the test strains was shown in Figure 2. A gradual increase in the LDH activity was recorded after 3 hrs and reaching the highest after 5 hrs of treatment. This indicated the MP -DCMf causes damage to the bacterial cell membrane. The statistical analysis revealed that LDH release had moderate positive correlation with the activity index and time of treatment whereas strong negative correlation with CFU count.The regression analysis also strongly supported the correlation results as represented in Figure 3.

Effect on the cell morphology

MP-DCMf caused cellular damage as confirmed by SEM photomicrographs (Figure 4A-D). It showed changes in the morphology and shape of the bacterial cells, like the formation of notch (4B), blebbing (4C) and decay of the cell walls (4D) which is comparable to the untreated bacteria (4A).

TLC analysis

The comparative TLC analysis of the MP-DCMf showed the presence of alkaloids, flavonoids, and phenols in the active fraction as shown in Figure 5.The presence of alkaloid in the fraction was confirmed as orange spots after spraying with the Dragendoff's reagent which had R_f value of 0.55. In flavonoids test (Fig. 5B) quercetin (spot 1), MP-DCMf (spot 2) and MP-DCMf + quercetin (spot 3) showed the same R_f values i.e. 0.254 (Fig. 5B, spot, whereas in phenol (Fig. 5A) gallic acid (spot 1), MP-DCMf (spot 2) and MP-DCMf + gallic acid (spot 3) showed the same R_f values of 0.690.

Table 1: Spectrum of antimicrobial activity of HJ-DCMfagainst the enterobacterial strains.

S. No.	Enteric Strains	Strains properties	Growth condition	DCM Fraction	Antibiotics (50 µg/mL)	Activity Index
1.	*B. cereus*	Pathogen of food borne illnesses	TSB, 37°C	15.6 ± 0.78	18.0 ± 0.9	0.866
2.	*B. subtilis*	Food borne illnesses	TSB, 37°C	15.0 ± 0.75	18.0 ± 0.9	0.833
3.	*E. coli*	GP, colicin indicator strain	TSB, 37°C	18.0 ± 0.9	20.0 ± 1	0.9
4.	*E. faecalis*	Gastrointestinal causing abdominal infections	NB, 37°C	14.8 ± 0.74	18.0 ± 0.9	0.822
5.	*L. monocytogenes*	HPF borne pathogen causing listerosis	BHI, 37 °C	15.0 ± 0.75	18.0 ± 0.9	0.833
6.	*S. aereus*	Pathogenic, antibiotic sensitive strain	TSB, 37°C	12.5 ± 0.63	18.5 ± 0.93	0.675
7.	*S. mutans*	Dental caries pathogen	BHI, 37 °C	17.0 ± 0.85	19.5 ± 0.98	0.871
8.	*S. typhimurium*	Gastroenteritis pathogenic prototrophic strain	TSB, 37°C	16.4 ± 0.82	19.0 ± 0.95	0.863
9.	*V. parahaemolyticus*	Gastroenteritis halophilic organism	NB, 37°C	14.6 ± 0.46	17.0 ± 0.85	0.858

Here, DCM solvent shows negative inhibition zone against all the enteric pathogens.

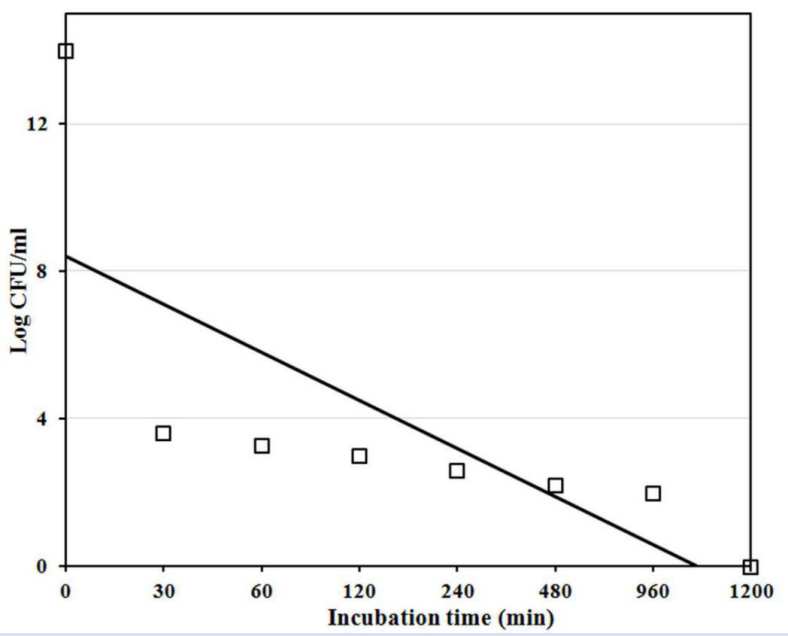


Figure 1: Effects of the MP-DCMf (3.12mg/ml) on viability and loss of cellular integrity of *E. coli* MTCC 723 as measured by quantitation of lactate dehydrogenase assay. Here, the values are the average of triplicate trials ± SE

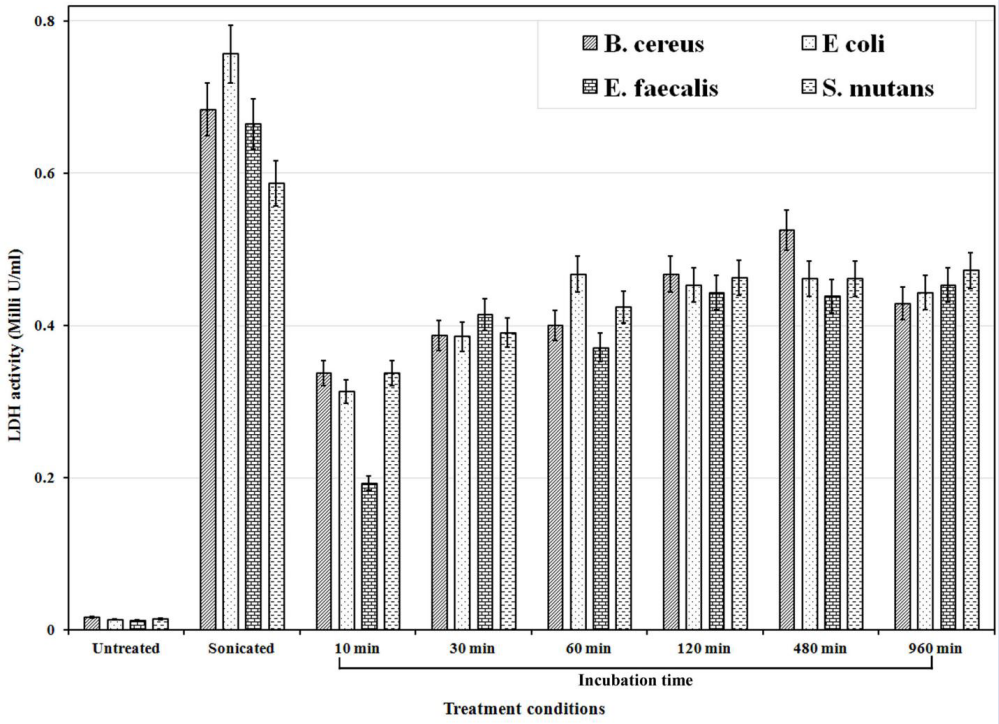


Figure 2: LDH activity of *B. cereus*, *E. coli*, *E. faecalis*, *S. mutans* induced by MP-DCMf (3.12mg/mL). Each point represented the mean of triplicate trials and error bars represented standard errors.

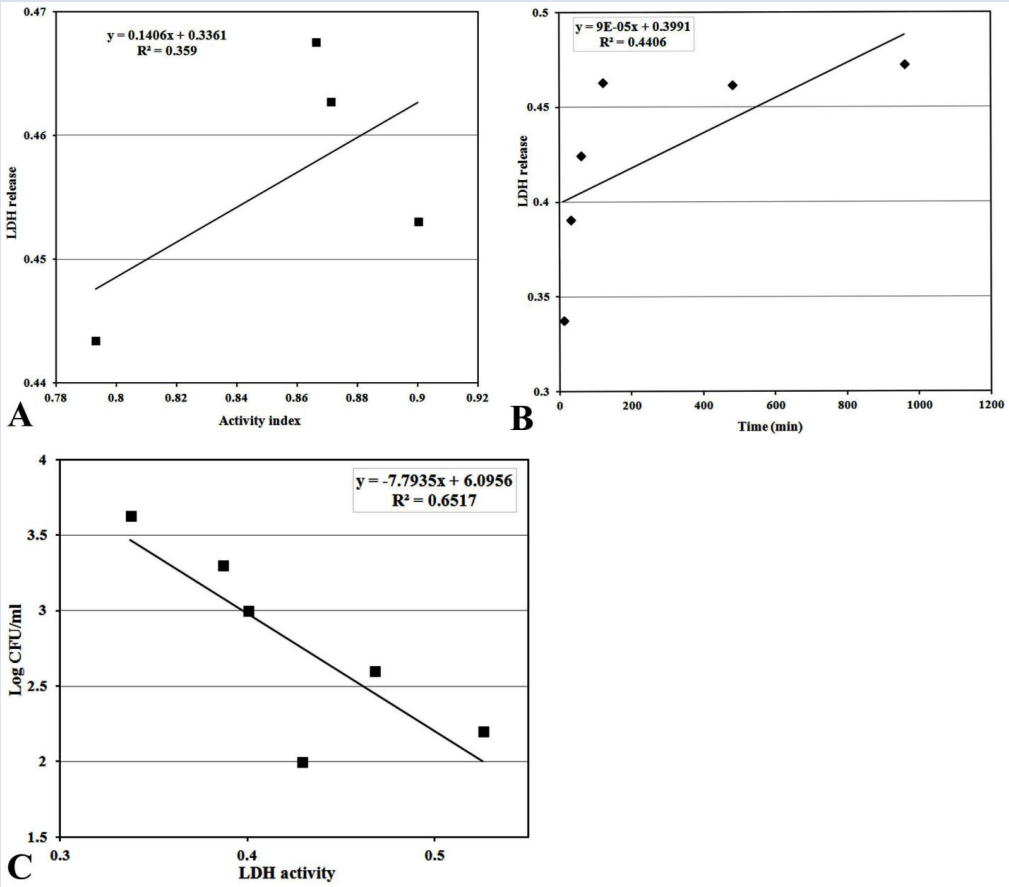


Figure 3: Correlation of LDH realese with different related parameters: (A) LDH and Activity index; (B) LDH and Treatment time; (C) LDH and log CFU/ml.

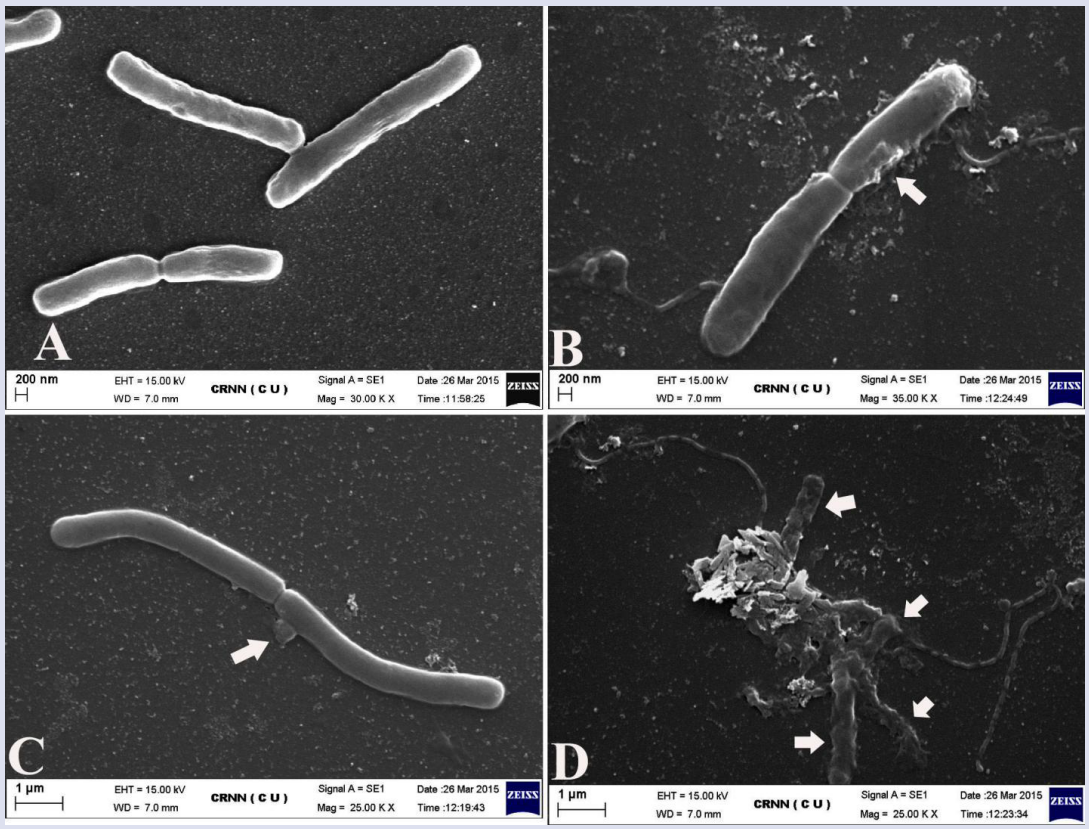


Figure 4: SEM photomicrogram of *L. monocytogenes* induced by the MP-DCMf. (A) Untreated cells; (B, C and D) Treated cells.

GC-MS analysis

The GC-MS analysis showed that D-Carvone occupied the maximum peak area (30.949%) and Cyclopentane, 1,1-dimethyl occupied the lowest peak area (0.024%). Other compounds present in significant proportions were 1H-Isoindole-1, 3(2H)-dione, 2-(2-chlorophenyl) (28.483%), D-Limonene (9.256%), Phosphonic acid (1.723%), and p-Cresol (0.551%). About twenty two terpenoid compounds that occupied 66.881% of total moles were identified. The first compound was Fampridine with the least retention time *i.e.*, 4.773 min. and the last compound was 1H-Isoindole-1,3(2H)-dione, 2-(2-chlorophenyl) with the highest retention time *i.e.*,19.189 min. as detected in the spectrum (Figure 6, Table 2).

DISCUSSION

MP-DCMf has potential broad spectrum antibacterial activity at a dose of 25 mg/ml against Gram-negative and Gram-positive bacteria with the average diameter of zone inhibition of 17.00 mm and 15.00 mm, respectively. The minimum and maximum MBC values (6.25 mg/ml and 1.56 mg/ml, respectively) were significantly lower than the MBC value of other species of *Hydrocotyle.*[16] Bactericidal activity of the MP-DCMf was the highest against the enteric pathogen *E. coli*, *S. enterica* serovar. *typhimurium* and another highly entero-pathogenic strain, *E. faecalis. B. cereus*, *B. subtilis, L. monocytogenes,* and *S. mutans* were also very susceptible to the active fraction at a lethal dose of 3.12mg/ml. The most of the bacterial strains showed their growth inhibition within the minimum concentration of 1.56mg/ml. Hence, the MP-DCMf fraction contains some potential bactericidal compounds that can be used against these pathogenic bacteria. The MP-DCMf also exhibited significant bactericidal activity against the dental carries pathogen, *S. mutans*. The antibacterial efficacy, MIC and MBC of the MP-DCMf was greater than the methanol extract[13] as shown in Table 3. LDH activity results indicated that the MP-DCMf causes extracellular leakage due to bacterial cell membrane damage. Time kill assay has the implication in the bactericidal kinetics of the antibacterial agent against specific bacterial strain. It demonstrates the degree of lethality of the particular antibacterial agent. The comparative TLC analysis shows the presence of some potent antibacterial alkaloids, phenols and flavonoids in the MP-DCMf (Figure 5).

GC-MS analysis of the MP-DCMf showed eight alkaloids, six phenolics, one flavonoid and one glycoside compounds present at a proportion of 30.126%, 3.579%, 0.055% and 0.075% of total moles, respectively as shown in the Table 2. A GC-MS study of *Hydrocotyle bonariensis* also reported the presence of monoterpenes (53.6% mol) and sesquiterpenes (10.5% mol) as the major phytochemical classes.[17] The monoterpenes α-pinene, β-pinene, and limonene represent the main components of the oil. These indicate the genus *Hydrocotyle* possesses the biosynthetic machinary of novel antimicrobials. For large scale isolation, purification and pharmaceutical application this plant may provide a valuable tool. Further *in-vitro* propagation and metabolic engineering could provide life supporting drugs for industrial uses.

In the MP-DCMf p-Cresol was detected as the major phenolic compound which possesses significant antibacterial potentiality and was also available in the methanol extract of the same plant.[18] It might also increase the plasma membrane permeability that results in higher leakage of fluid material from bacterial cells and inhibit microbial respiration.[19] Iso-indole alkaloids possess good activity against the Gram-negative bacteria (*E. coli, K. pneumonia* and *P. aeruginosa,*) and yeasts (*C. albicans, S. cerevisiae*) with MIC ranging from 50-200µg/ml, while it was less susceptible against the Gram-positive bacteria (*B. megaterium, B. subtilis* and *S. aureus*). Iso-indole alkaloids act as nucleic acid synthesis inhibitor.[20] Terpenoid shows antibacterial activity against *E. coli, E. faecalis*, *K. pneumonia, P. aeruginosa* and *S.*

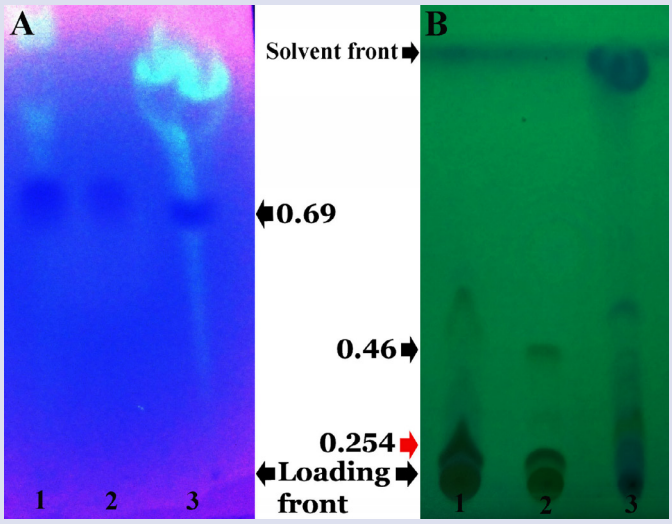


Figure 5: Comparative TLC analysis of the MP-DCMf. (A) Phenols and (B) Flavonoids.

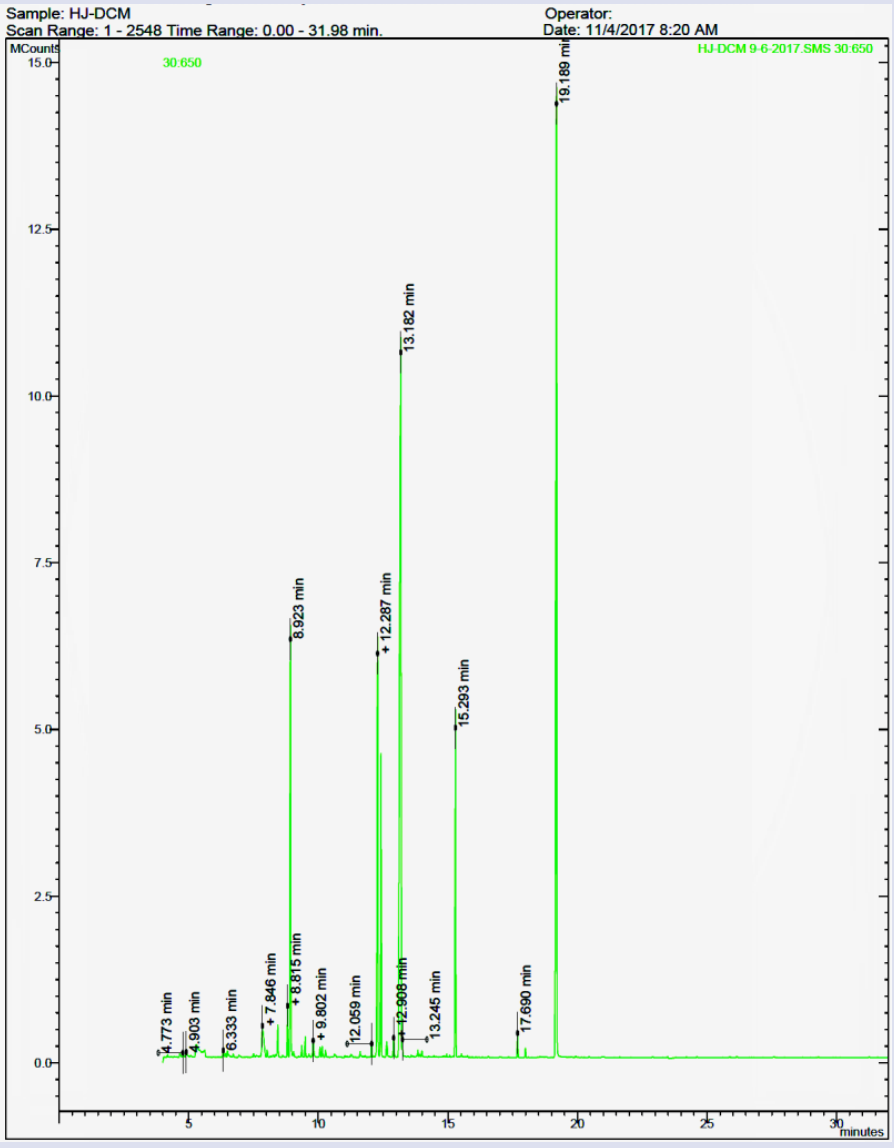


Figure 6: Gas chromatogram of the MP-DCMf.

Table 2: Chemical nature of the phytocompounds detected in GC-MS.

Class of compounds	Name of the compounds	Total Mole %
Alkaloids	(i) Fampridine; (ii) 3-Furaldehyde; (iii) Pyridine-2,6-dimethyl;(iv) Pyrazine,2,3-dimethyl; (v) Pyrazole-4-carboxaldehyde,1-methyl-; (vi) Pyrazine, 2-ethyl-6-methyl; (vii) 3,6-Dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran; (viii) 1H-Isoindole-1,3(2H)-dione, 2-(2-chlorophenyl).	30.126
Flavonoids	Ethanone, 1-(1,3a,4,5,6,7-hexahydro-4-hydroxy-3,8)	0.055
Terpenoids	(i) 2-Cyclopenten-1-one,2-methyl;(ii) Trans-beta-Ocimene; (iii) 2-Cyclopenten-1-one,3-methyl; (iv) Alpha-Phellandrene; (v) 2,4,4,6-Tetramethyl-6-phenylheptane; (vi) D-Limonene; (vii) 2-Cyclopenten-1-one, 2,3-dimethyl; (viii) 3-Carene; (ix) Ethanone, 1-(1H-pyrrol-2yl); (x) Cyclopentane,1,1dimethyl; (xi) Benzene,(butoxymethyl), (xii) 4-(1,2-Dimethyl-cyclopent-2-enyl)-butan-2-one; (xiii) 2Cyclopentene-1-butanal,gamma2,3tetramethyl; (xiv) Cyclohexanone , 2-methyl-5-(1-methylethen) trans; (xv) Cyclohexanone, 2-methyl-5-(1-methylethenyl)-, trans; (xvi) Cyclohexanol, 2-methyl-5-(1-methylethenyl)-trans; (xvii) D-Carvone, (xviii) 2-Cyclohexen-1-one,2-methyl-5-(1-methylethyl); (xviv)Thymol;(xvv)1,6-Octadien-3-ol,3,7-dimethyl-,formate; (xvvi) 2,6-Octadien-1-ol, 3,7-dimethyl-, acetate; (xvvii) Benzene, 1,2,3-trimethoxy-5-(2-propenyl).	66.881
Phenol	(i) Phosphonic acid, (p-hydroxyphenyl); (ii) p –Cresol; (iii) Mequinol; (iv) 2,4-Dimethyl phenol; (v) p-Menth-1(7)-en-9-ol; (vi) 1,3-Benzodioxole, 4-methoxy-6-(2-propenyl).	3.579
Glycosides	1H-Pyrrole-2-carboxaldehyde	0.075

Table 3: Comparative analysis of antibacterial potentiality of both DCM and Methanol extracts.

S. No.	Name of micro-bial strains	Diameter of zone inhibition		MIC value		MBC value	
		DCM extract (25mg/ml)	Methanol extract (324 mg/ml)	DCM extract (mg/ml)	Methanol extract (mg/ml)	DCM extract (mg/ml)	Methanol extract (mg/ml)
1.	*B. cereus*	15.6 ± 0.78	15.00 ± 0.75	0.78 ± 0.04	20.25 ± 1.01	1.56 ± 0.08	40.50 ± 2.03
2.	*E. coli*	18.00 ± 0.9	17.00 ± 0.85	0.78 ± 0.04	40.50 ± 2.03	3.12 ± 0.16	81.00 ± 4.05
3.	*L. monocytogenes*	15.00 ± 0.75	16.00 ± 0.80	8.60 ± 0.43	40.50 ± 2.03	3.12 ± 0.16	81.00 ± 4.05
4.	*S. typhimurium*	16.4 ± 0.82	20.00 ± 1	10.50 ± 0.53	20.25 ± 1.01	1.56 ± 0.08	40.50 ± 2.03
5.	*S. aereus*	12.5 ± 0.63	18.00 ± 0.9	3.12 ± 0.16	81.00 ± 4.05	6.25 ± 0.31	81.00 ± 4.05
6.	*S. mutans*	17.00 ±0.85	12.00±0.6	1.56 ± 0.8	5.80 ± .29	3.12 ± 0.16	20.25 ± 1.01

aureus.[21] Thymol was detected as the major terpenoid compound in the tested sample. Thymol, a phenolic monoterpenoid having hydroxyl groups at the different positions of its phenolic ring disrupts the cell membranes by interaction with the membrane proteins of the bacterial cell.[22] This loss of cellular integrity and decay of the cell walls due to active compounds present in the DCM fraction was visualized by the SEM study. Statistical analyses validated the antibacterial phenomena indicating the active compounds had a very strong bactericidal activity. The activity index and LDH correlation analysis proved that the bacterial zone inhibition by the active fraction is positively correlated with LDH release through the cell membrane of the bacterial cell. The Dosha Karma (Effect on humor) of Mandukaparni is that it overcomes Pitta dosha and Kapha dosha.[23] Our present study specified its effect on Pitta dosha by combating the gastrointestinal infections and related diseases of the hepato-biliary system.

CONCLUSION

The study suggested that the MP-DCMf contained some potent anti-bacterial compounds belonging to the class alkaloids, monoterpenoid and phenol. The antibacterial efficacy of these three types of secondary metabolites among which the alkaloids have specific for one or a limited number of molecular targets whereas phenols are multi-target agents modulating the activity of proteins, nucleic acids and bio-membranes in a less specific way. Thus, it can be concluded that the Mandukaparni (*H. javanica*) which is used in ayurvedic preparations have the potential to treat against bacterial pathogens of gastrointestinal infections, food borne and topical diseases (including dental caries pathogens).This is the first scientific report on the antibacterial potentiality of DCM fraction of the high altitude folklore medicinal herb, *H. javanica*. So, further isolation, purification and structure elucidation of the active compounds, their acute as well as cytotoxicity tests, QSAR guided chemical study for drug development, and vivid mode of action might explore the better application of this novel repository herb for anti-enteric drug development.

ACKNOWLEDGEMENTS

The corresponding author is grateful to DST, Government of India (SERB/F/5369/2013-'14) for partial financial help of the study. The authors are also thankful to the KIIT-TBI, KIIT University, Bhubaneswar, India for GC- MS analysis and CRNN, University of Calcutta, Kolkata, India for SEM study.

CONFLICTS OF INTEREST

None.

ABBREVIATIONS

GC-MS: Gas Chromatography-Mass Spectrometry; DCMf: Dichloro Methane Fraction; SEM: Scanning Electron Microscope; MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration; TLC: Thin-Layer Chromatography; CFU: Colony-Forming Unit; LDH: Lactate Dehydrogenase.

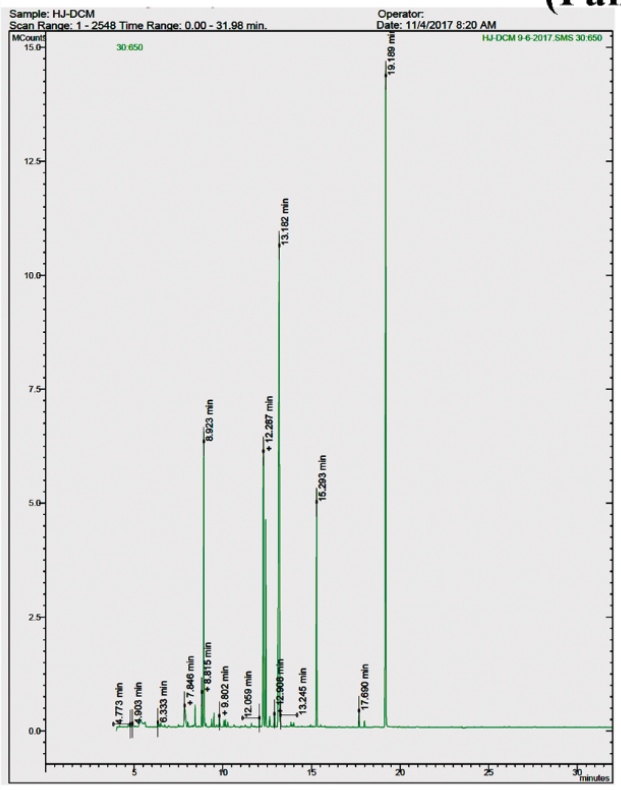
REFERENCES

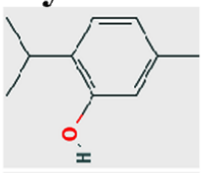
1. Khare CP. Ed. *Hydrocotyle javanica*Thunb. In: Indian medicinal plants. Springer, New York, 2007.
2. Diwan PC, Karwande I, Singh AK. Anti-anxiety profile of mandukparni *Centella asiatica* Linn in animals. Fitotera. 1991;62:255-7.
3. Orhan IE. *Centella asiatica* (L.) Urban: From traditional medicine to modern medicine with neuroprotective potential. Evid Based Comp Alt Med. 2012;2012:946259.
4. Krithika N, Arumugasamy K. Toxicity and antipyretic effects of ethnomedicinal plant *Hydrocotyle javanica* Thunb. (Apiaceae). World J Pharm Pharm Sci. 2016;6(01):1468-80.
5. Srinivasan D, Nathan S, Suresh T, Lakshmana Perumalsamy P. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. J Ethnopharm. 2001;74:217-20.
6. Mandal M, Misra D, Ghosh NN, Mandal V. Physicochemical and elemental studies of *Hydrocotyle javanica* Thunb. for standardization as herbal drug. Asi Pac J Trop Bio-med. 2017;7(11):979-86.

7. Kumar N, Chaubey S, Singh SK, Tewari RC. Comprehensive literature review of Mandukparni (*Centella asiatica*) w.s.r. to its medicinal properties. Int J Ayur Pharma Res. 2017;5(5):65-71.
8. 5th Programme Report, Programme for control of diarrhoeal diseases. Vol. 63. Geneva: WHO Bull; 1985. WHO; pp. 557-772.
9. Park K. Acute diarrhea diseases. In: Park K, editor. Parks textbook of preventive and social medicine, 18th ed. Jabalpur: Banarasidas Bhanot Publishers, 2005. 171-74.
10. Ahmad I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. J Ethnopharm. 1998;62:183-93.
11. Sodipo OA, Wannang NN. Ethnopharmacological survey of plants used by Trado-Medical Practitioners (TMPs) in the treatment of typhoid fever in Gomari airport ward, Jere local government area, Borno State, Nigeria. Amer J Ethnomed. 2015;2(4):185-218.
12. Patwardhan B, Warude D, Pushpangadan P, Bhatt N. Ayurveda and traditional Chinese medicine: A comparative overview. Compl Alter Med. 2005;2:465-73.
13. Mandal M, Paul S, Uddin MR, Mandal MA, Mandal S, Mandal V. *In vitro* antibacterial potential of *Hydrocotyle javanica* Thunb. Asia Pac J Trop Dis. 2016; 6: 54-62. https://doi.org/10.1016/S2222-1808(15)60985-9
14. Misra D, Mandal M, Ghosh NN, Mandal V. Pharmacognostic standardization of an ethnomedicinal aquatic herb, *Monochoria hastata* (L.) Solms for its antibacterial potentiality. Pharmacog J. 2018;10(3):533-40.
15. Pathak S, Jana B, Mandal M, Mandal V, Ghorai TK. Antimicrobial activity study of a μ3-oxo bridged $[F_{e3}O(PhC_{O2)6}(MeOH_{)3}](N_{O3})(MeOH_{)2}]$ cluster. J Mol Str. 2017;1147:480-6.
16. Eduardo V, de Dios MJ, Isidro H, Analía D, Christian S. Antimicrobial activity of dichloromethane extracts of eleven plants from the flora of Entre Ríos (Argentina). Pharmacol Online. 2006;3:845-9.
17. Huong TNL, Phung KN, Suong NN. Chemical examination of *Hydrocotyle bonariensis* L. (Apiaceae). J Chem. 2009;47(5):542-6.
18. Namasivayam K, Karuppanan A, Habibulla AK. Rangasamy N. GC-MS profiling of ethnomedicinal plant *Hydrocotyle javanica* Thunb. Ori J Chem. 2017;33(4).
19. Rao KN, Venkatachalam SR. Inhibition of dihydrofolate reductase and cell growth activity by the phenanthroindolizidine alkaloids pergularinine and tylophorinidine: the in vitro cytotoxicity of these plant alkaloids and their potential as antimicrobial and anticancer agents. Toxic *In Vitro.* 2000;14:53-9.
20. Lozykowska SK, Kędzia B, Karpińska E, Bocianowski J. Microbiological activity of caraway (*Carum carvi* L.) essential oil obtained from different origin. Acta Scient. 2013;35(4):495-500.
21. Zengin H, Baysal HA. Antibacterial and antioxidant activity of essential oil terpenes against pathogenic and spoilage-forming bacteria and cell structure-activity relationships evaluated by SEM microscopy. Molecul. 2014;19:17773-98.
22. Wink M. Modes of action of herbal medicines and plant secondary metabolites. Medi. 2015;2:251-86.
23. Ashalatha M, Shenoy LN. A review on Mandukaparni. Int Ayur Med J. 2016;4(02):129-40.

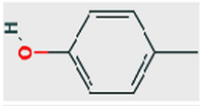
GRAPHICAL ABSTRACT

Mandukaparni (*Hydrocotyle javanica* Thunb.)
(Family Araliaceae)

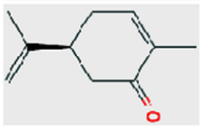




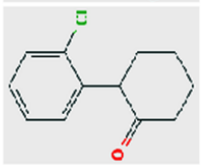
D-carvon



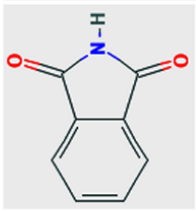
p-Cresol



Thymol

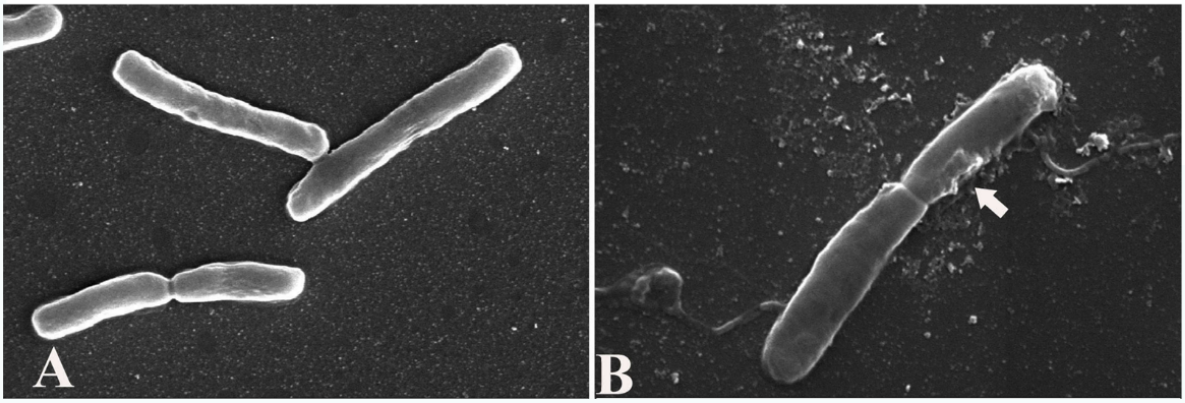


2-(2-chlorophenyl) Cyclohexanone



1H-Isoindole-1,3 (2H)-dione

GC-MS profiling



Anti-enterobacterial potentiality

ABOUT AUTHORS

Dr. Manab Mandal did his Ph. D. research work on antibacterial potential of *Hydrocotyle javanica* Thunb. from the Department of Botany, University of Gour Banga, Malda. He is currently engaged as Assistant Professor of Botany in Dukhulal Nibaran Chandra College, Aurganbad, W.B., India.

Dr. Debabrata Misra did his Ph. D. research work on antibacterial potential of Monochoria hastata (L.) Solms against the gastrointestinal micro flora from the Department of Botany, University of Gour Banga, Malda in West Bengal. He is currently working as Head of a Govt. sponsored Institution, Malda, W.B.

Mr. Narendra Nath Ghosh is a Ph. D. Research Scholar at the Department of Chemistry, University of Gour Banga, Malda. His research focuses on the theoretical and computational chemistry of fluorescent dyes.

Dr. Sukhendu Mandal is a Professor of Microbiology, University of Calcutta, W.B. His main focus of research is on drug-molecule interaction in the microbial system.

Dr. Vivekananda Mandal is a Professor of Botany, Department of Botany, University of Gour Banga, Malda. His main focus of research is on isolation and characterizaion antimicrobial secondary metabolites and their mode of action.

Cite this article: Mandal M, Misra D, Ghosh NN, Mandal S, Mandal V. GC-MS Analysis of Anti-Enterobacterial Dichloromethane Fraction of Mandukaparni (*Hydrocotyle javanica* Thunb.) – A plant from Ayurveda. Pharmacogn J. 2020;12(6)Suppl:1494-503.