Characterization and Antimicrobial Activity of Centella Asiatica

S. Agneeswari, S. Amutha, J. Jaya Arputha Jenishini

Abstract*: India is taken into consideration because the motherland of medicinal plants. Centella asiatica (L.) incorporates effective pharmocological compounds with antibacterial, antiinflamatory, anticancer and antioxidant belongings. In our have a look at it is pronounced that the fuel chromatography-mass spectrometry analysis revealed the presence of fifty compounds in C1 and 86 compounds in C2 and many of these compounds like tert-Butyl (five-isopropyl-2-methylphenoxy) dimethylsilane, silicic acid, diethyl bis(trimethylsilyl) esterhave antimicrobial property. Hence, the antimicrobial pastime become further analysed using distinct solvent extracts and ethanol extract was observed to be a terrific antimicrobial agent. The MIC of etanol extract in opposition to micro organism is decided as 0.A hundred twenty five µg/ml and the cell demise of bacteria is studied via DNA cleavage evaluation. This examine endorse the bioactive components of Centella asiatica (L.) leaf extract is a superb antimicrobial agent.

Keywords: Centella asiatica (L.), Antimicrobial, medicinal, characterization

I. INTRODUCTION

Plants are a rich supply of secondary metabolites with natural activities. Centella asiatica (L.) is a prostrate, faintly aromatic, stoloniferous, perennial, creeper herb, attains pinnacle as much as 15cm (6inches) and thrives significantly in shady and wet locations such as paddy fields, river banks forming a dense green carpet (Devkota 2009). A huge form of medicinal plant components are used to extract as raw pills and they own diverse medicinal houses. While a number of those raw tablets are gathered in smaller portions by using manner of the neighborhood organizations and plenty of various raw drugs are accrued in large quantities for many herbal industries (Uniyal 2006). The lively requirements of many tablets determined in vegetation are secondary metabolites. The antimicrobial sports activities of plant extracts may additionally reside in unique components which includes aldehyde and phenolic compounds (Lai 2004).

Screening of phytochemicals serves because the initial step in predicting the forms of capability energetic compounds from plant life (Chew 2011).The vital goal of this examine become to evaluate and observe the bioactive compounds and to observe the in vitro antimicrobial interest and DNA cleavage effect of the two carefully related Centella species based on morphological characters (Leaf duration) and specific bioactive compounds with exclusive solvent extraction.

Revised Manuscript Received on July 22, 2019.

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II. MATERIALS AND METHODS

Collection of plant material:

The leaves of *Centella asiatica* were accrued from Gnarode village of Kanyakumari District, Tamilnadu, India. It became identified and authenticated by way of Dr. P. Nagerndra Prasad, Head, Department of Biotechnology, Sri Paramakalyani College, Alwarkurichi, Tirunelveli. The plant changed into grouped into agencies based totally on the leaf length. The Centella asiaticaplant with small leaf size is grouped as C1 and big leaf size is grouped as C2 based totally on Morphology.

Preparation of plant extract:

The plant leaves were wiped clean and color dried. The dried leaves had been reduced to best particles the use of dry blender. Then the powdered leaves were extracted successfully with three unique solvents specifically ethanol, chloroform and aqueous by means of the use of soxhlet equipment and the extraction was accomplished for 24 hours at temperature primarily based on solvents. The extract changed into filtered and concentrated at 45°C the use of rotary vacuum evaporator and the extract turned into used for in addition research.

FTIR

Fourier Transform Infrared Spectroscopy (FTIR) Analysis. The specific extracts of Centellaasiatica turned into analyzed by FTIR (FTIR BRUKER ALPHA-E) to know the distinctive practical agencies gift within the plant extracts. The dried crude extract become subjected to analyze in FTIR wherein the diffuse reflectance technique was followed. The crude dried pattern was mixed with potassium bromide (KBr) to shape a very exceptional powder. This powder turned into then compressed into a thin pellet which changed into analyzed. KBr become also transparent in infrared mild. The samples have been irradiated by means of a wide spectrum of infrared light and the extent of absorbance at a particular frequency became plotted after Fourier transformation of the data.

The resulting spectrum became function of the natural molecule present in the sample. The absorbance turned into measured at 4000-four hundred nm for the identity and quantification of natural species. Compounds contained in the extracts were diagnosed in line with mounted criteria of Nyquist and Kagel, (1997).

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

GC-MS evaluation of the pattern became done using a Shimadzu GCMS-QP2010 gasoline chromatographmass spectrometer interfaced with a Turbo Mass quadrupole mass spectrometer, geared up with an Rtx-5 fused silica capillary column. The oven temperature turned into programmed from a hundred°C to 320°C at one hundred°C/min and a keep for 10 mins. Helium changed into used as carrier gasoline at go with the flow 1.Zero ml/min. The injector temperature become 250°C, injection size 1 µl neat, with split ratio 1:10. The interface and MS ion deliver had been maintained at 320°C and two hundred°C respectively and the mass spectra had been taken at 70eV with a mass take a look at variety of forty-seven-hundred amu (atomic mass unit). Data managing emerge as performed the use of GCMS solution software with modified approach of (Senthil 2016).

Antimicrobial activity

Antibacterial pastime of Centella asiatica was determined through disc diffusion technique on Muller Hinton agar (MHA) medium [22-24]. The Muller Hinton agar plate became organized and the inoculums have been spread on solid plates with sterile swab moistened with the bacterial suspension. 25 µl of 25 µg, 50 µg, 75 µg and one hundred µg of flower extract, 25 µl of double distilled water as poor control and streptomycin as tremendous manage become used. The check organisms used in this investigation consist of: Staphylococcus aureus, Bacillus subtilis, Streptococcus mutans, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Aspergillus flavus, Penicillium notatum and Rhizopus sp. Gifted by Inbiotics, Nagercoil. The bacterial isolates were cultured in a nutrient broth (Himedia, Mumbai, India) medium and incubated at 37°C for 18 hours. Streptomycin (25 mg, Hi-media) changed into used as advantageous manipulate for the bacterial traces and Millipore water become used as poor manage**.**

Determination of Minimum Inhibitory Concentration (MIC)and Minimum Bactericidal Concentration (MBC)

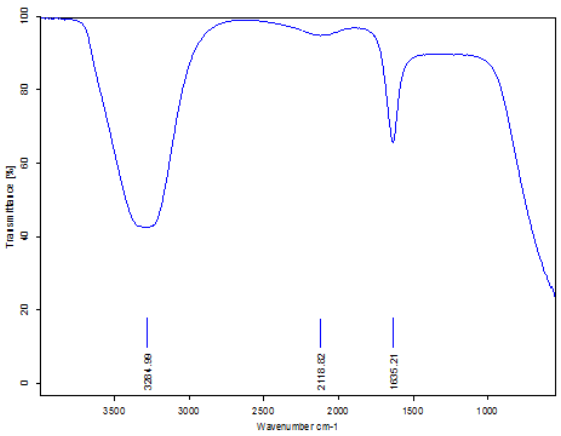
Ten sterile tubes had been located in a rack and have been categorized. The MIC of the extracts changed into decided through way of diluting the diverse concentrations (1, 0.Five, 0.25, zero.One hundred twenty five, 0.062, zero.031, zero.Half of and zero.007 mcg/ml). Equal extent of the nutrient broth became jumbled together first eight tubes. Specifically, 0.1ml of standardized inoculums of one to two x 107cfu/ml changed into brought to each eight tube. The tubes had been incubated aerobically at 37°C for 18-24hours. Two manage tubes have been maintained for every take a look at batch. This is as follows: Ninth tube containing extracts and the boom medium with out inoculums (antibiotic manage) and the 10th tube containing the boom medium, physiological Nutrient broth and the inoculums (organism manage). MIC have become decided as the lowest interest of the extracts permitting no visible growth (no turbidity) even as as compared with the control tubes. The MBC emerge as determined with the resource of subculturing the test dilution on glowing solid medium and in addition incubated at 37°C for 18-24hours. The lowest interest of MIC tubes and now not the use of a seen bacterial growth on stable medium have become appeared as MBC (Akinyem 2006).

Cleavage of pUC18 DNA

The cleavage of supercoiled pUC18 DNA to its nicked round form studied with the aid of way of the use of agarose gel electrophoresis. PUC18 DNA (0.Three µg) dissolved in five mmolL-1Tris-HCl/50 mmolL-1NaCl buffer (pH 7.2), modified into treated with the complexes. The combination grow to be incubated at 37□ C for 1 hour and then mixed with the loading buffer containing 25% bromophenol blue, zero.25% xylene cyanol and 30 % glycerol. Each sample (10-three M, zero.Five µL) was loaded into 1% (w/v) agarose gel. Electrophoresis become undertaken for 2 h at 100 V in Tris-acetate-EDTA (TAE) buffer (pH eight.0). The gel modified into stained with ethidium bromide for five minutes after electrophoresis after which photographed under a UV transilluminator. To beautify the DNA cleaving hobby of the complexes, hydrogen peroxide (a hundred µmolL-1) became brought to every sample.

IV.RESULTS

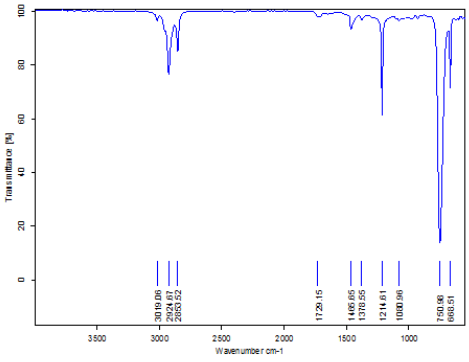
FTIR analysis of *Centella asiatica*leaf extract



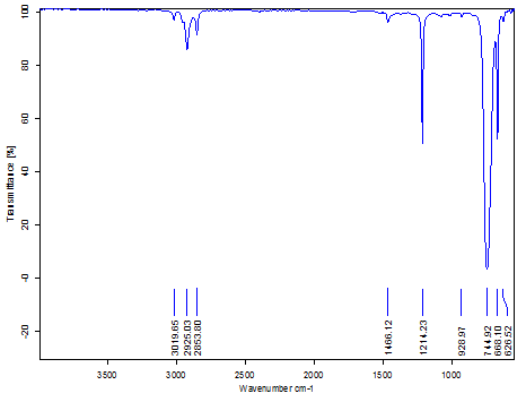
(a)

Structural elucidation of the extract become executed by FTIR analysis. The aqueous extracts of C1 confirmed two spectrum, high extensive top at 3284 cm-1 and confirmed the presence of alkynes, carboxylic acids, phenols, amides and amines with stretches O-H, dimer OH, Aro, H-H bond, C=O stretch and NH2 bond. Chloroform extracts confirmed excessive height at 744 cm-1 with aromatics C-H stretch.

Other peaks 668 cm-1, 2925cm-1 and 2853 cm-1 includes alkynes, alkanes, carboxylic acids, alkyl halides, amines, esters, aromatics with C-H, dimer OH, C-N and C-O stretch. The ethanol extract confirmed greater quantity of peaks the high sharp peaks at 1087 cm-1 and 1044 cm-1 confirmed the presence of alkyl halides, ethers, amine, carboxylic and esters with C-F, C-O, C-N and N-H stretch. Medium and occasional peaks along with 3332cm-1, 2973cm-1, 1379cm-1 and 879cm-1 have been additionally found where the C2 trait of Centellaasiatica shows same peaks as in C1 and the ethanol extract reveals a few miscellaneous group which include thiocarbonyl and phosphine with C=S, P-H and Si-oR bonds.



(b)



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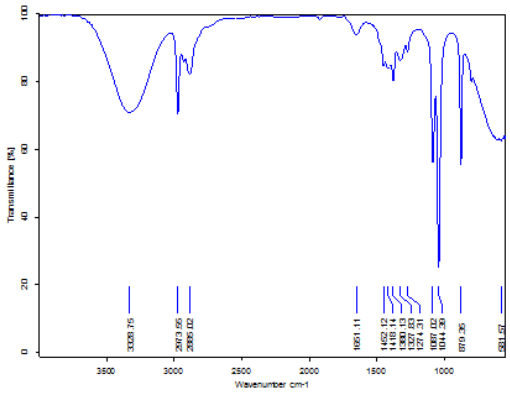

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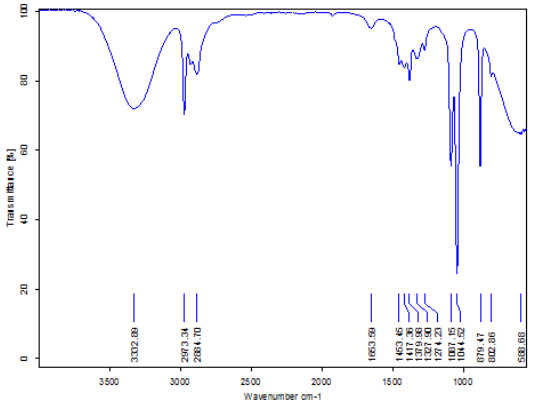

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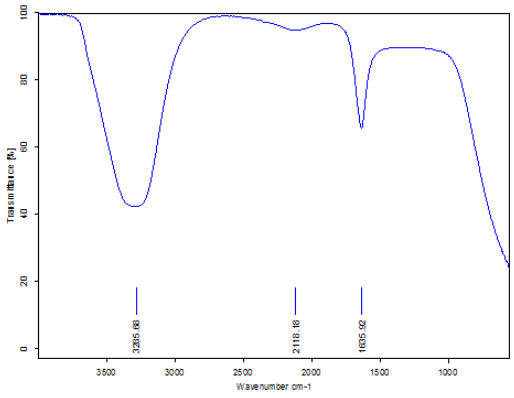


(a)

Identification of various compounds by GCMS analysis

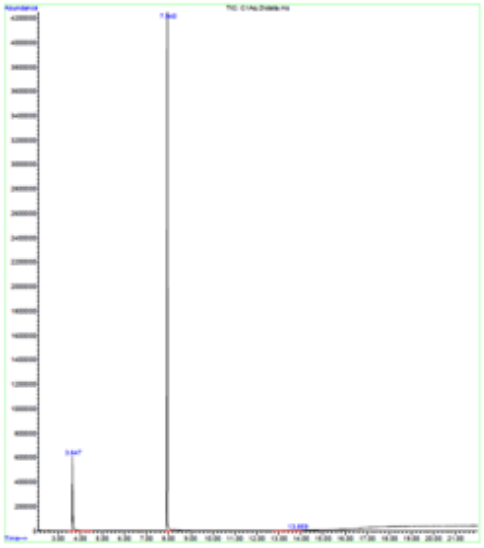


Figure 7: GC-MS chromatogram of aqueous extract of *Centella asiatica* (C1)leaves.

The diverse phytocomponents of C1 and C2 with different solvent extracts were identified from GCMS chromatogram (Figure 7, 8, nine, 10, 11 and 12).

C1 trait of Centellaasiatica showed the presence of compounds like Silane, methyltripropyl-, Phosphonous diiodide, (trifluoromethyl)-, (CH3)2NCl, Benzene, isocyanato, Bicyclo[3.1.1,]hept-2-ene-2-methanol, 6,6-dimethyl-, three-Isoxazolamine, five-methyl-, Diethyl Phthalate, Acetamide, N-(4-fluorophenyl)-2,2,2-trifluoro-, 3,5-Ethanoquinolin-10-one, decahydro-1,7-dimethyl-, [3R-(3.Alpha.,4a.Beta.,5.Alpha.,7.Beta.,8a.Beta.)]-, n-Heptadecanol-1, 6-Nitro-1H-quinazoline-2,four-dione, (5S,6aR,10aS)-5-Propyldecahydrodipyrrolo [1,2-a:1',2'-c]pyrimidine and Anthranilic acid, N-methyl-, butyl ester in Ethanol, Methyl salicylate and Diethyl Phthalate in Chloroform and Methyl salicylate, Diethyl Phthalate and 5-Acetamido-4,7-dioxo-4,7-dihydrobenzofurazan in aqueous. These compounds are tabulated with their molecular weight, structure and biological sports in Table 1. The C2 trait of Centellaasiatica showed the presence of Ethanethioamide, N,N-dimethyl-, Benzene, 1-(1-buten-three-yl)-four-pentyl-, tert-Butyl(5-isopropyl-2-methylphenoxy)dimethylsilane, Dodecahydropyrido[1,2-b]isoquinolin-6-one, Silicic acid, diethyl bis(trimethylsilyl) ester, Arsenous acid, tris(trimethylsilyl) ester, Quinoline, 2-chloro-6-methoxy-4-methyl-, N-Methyl-1-adamantaneacetamide and N-Methyl-1-adamantaneacetamide in ethanol, Methyl salicylate and Diethyl Phthalate in chloroform and Methyl salicylate, o-aminobenzohydroxamic acid, Diethyl Phthalate and 1,2-Bis(trimethylsilyl)benzene in aqueous extract.

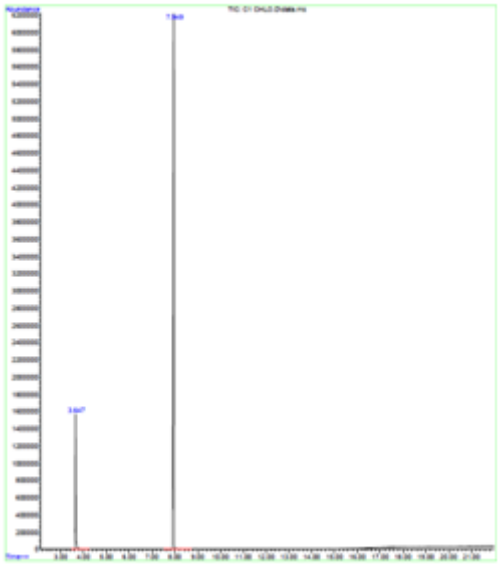


Figure 8: GC-MS chromatogram of chloroform extract of *Centella asiatica* (C1)leaves

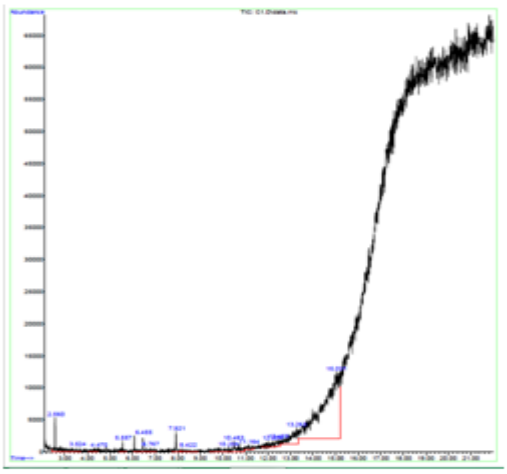


Figure 9: GC-MS chromatogram of ethanol extract of *Centella asiatica* (C1)leaves

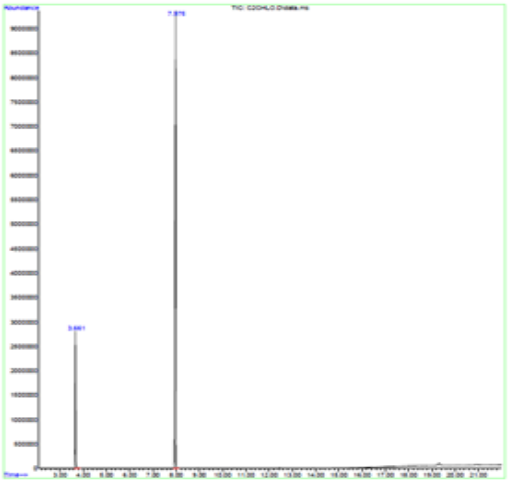


Figure 11: GC-MS chromatogram of chloroform extract of *Centella asiatica* (C2)leaves

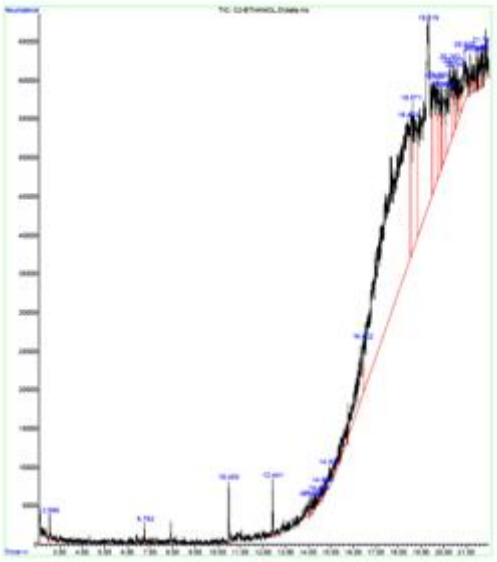


Figure 12: GC-MS chromatogram of ethanol extract of *Centella asiatica* (C2)leaves

Antimicrobial activity

The special solvent extract ofCentellaasiatica (C1 and C2) have been examined towards diverse bacterial and fungal traces for its antimicrobial pastime are shown in Table 1 and a couple of. Ethanol extract of C1 and C2 indicates boom inhibition against all bacterial and fungal traces tested the ethanol extract of C1 inhibits bacteria E.Coli with maximum zone of inhibition 21mm and it inhibits fungal pressure Aspergillus flavus with 20 mm. Whereas, C2 ethanol extract suggests hobby in opposition to Pseudomonas aeruginosa with most inhibition sector 18 mm. Aqueous extract of C1 and C2 have been completely inactive against maximum of the stains and determined to be least energetic towards some. However, the chloroform extract of each C1 and C2 trends of Centellaasiaticashows mild impact at the tested lines (Figure 13, 14 and 15). The values inside the tables are expressed as imply ± SD (standard deviation). It become proven that the ethanol extract confirmed proper bactericidal property and has maximum inhibition towards E.Coliand the minimal inhibitory attention (MIC) is 0.125 µg/ml and minimum bactericidal awareness (MBC) also confirmed

Table 1: Antibacterial activity of different solvent extract of *Centella asiatica* (C2)leaves

Samples	Strains (mm)								
	S. aureus	*B. subtilis*	*S. mutans*	*E.coli*	*K. pneu*	*P. aeru*	*Aspergillus flavus*	*Penicillium notatum*	*Rhizopus sp.*
C2.E	17	14	13	14	16	18	8	13	11
C2.Aq	-	10	-	-	10	10	-	-	-
C2.Chlo	8	13	11	10	14	13	10	8	8
PC	13	16	18	18	20	16	13	12	17
NC	-	-	-	-	-	-	-	-	-

Keys:

S. aureus - *Staphylococcus aureus (Gram + ve)*
B. subtilis - *Bacillus subtilis (Gram + ve)*
S. mutans - *Streptococcus mutans(Gram + ve)*

K. pneumoniae - *Klebsiella pneumoniae (Gram –ve)*
E.coli - *Escherichia coli(Gram –ve)*
P.aeruginosa - *Pseudomonas aeruginosa(Gram–ve)*

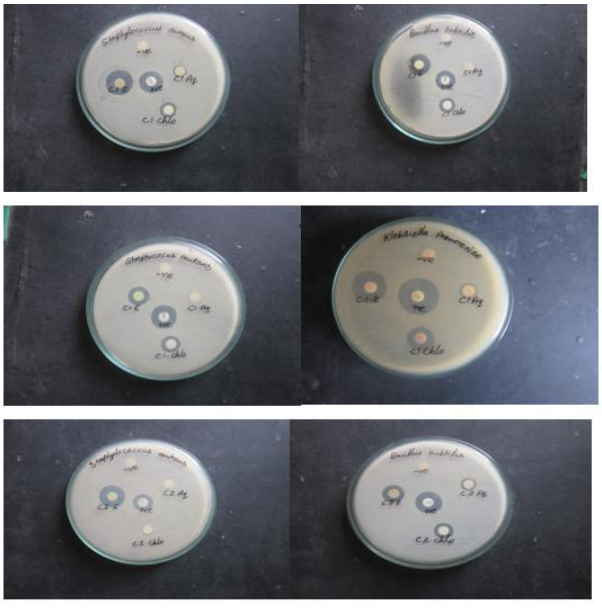


Figure13: Antibacterial activity of different solvent extract of*Centella asiatica* (C1 and C2)leaves against Gram positive bacteria

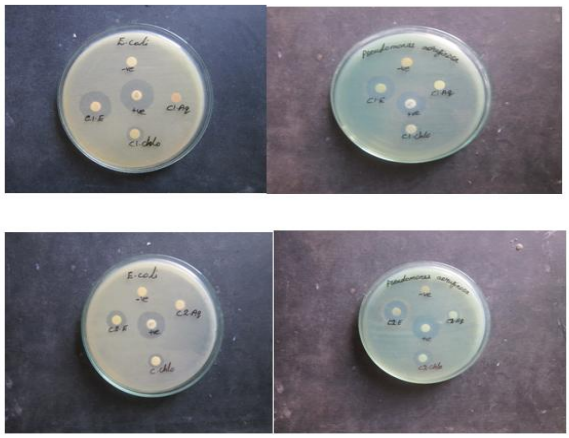
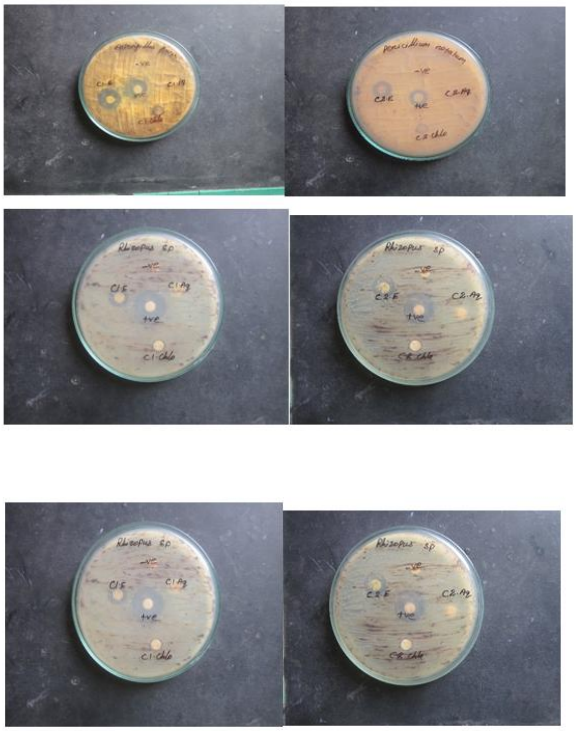


Figure14: Antibacterial activity of different solvent extract of*Centella asiatica* (C1 and C2)leaves against Gram negative bacteria



DNA Cleavage

The effect of Centella asiaticaleaf extract on chromosomal DNA cleaving became detected invitro the use of agrose gel electrophoresis .

The degradation of DNA is visualised in gel with ehtidium bromide stain as bands (Figure 16). The DNA remains as bands simply beneath the wells cleaved simplest few sites and denoted as weak degradation. The aqueous and chloroform extract of C1 and C2 shows no clevage (Lane 2,3,5 and 6). The ethanol extractof C1 and C2 dealt with cells showed fragments and the degradation become visualised as a smear (Lane four and seven) .

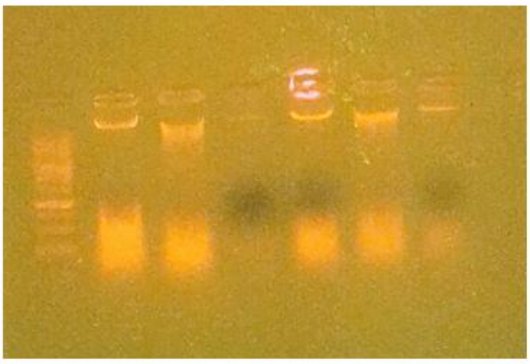


Figure16: Cleavage of pUC18 DNAby different solvent extract of*Centella asiatica* C1 and C2

Lane 1: Marker DNA,Lane 2: Plasmid DNA + C1 Aqueous, Lane 3: Plasmid DNA + C1 Chloroform

Lane 4: Plasmid DNA + C1 Ethanol,Lane 5: Plasmid DNA + C2 Aqueous

Lane 6: Plasmid DNA + C2 Chloroform Lane 7: Plasmid DNA + C2 Ethanol

V.DISCUSSION

In this take a look at we investigated diverse bioactive compounds of Centella asiatica leaves with awesome morphology (leaf period). Based on preceding have a examine the morphological style of the leaf can decide the genetic afflinities amongst and inside genus (Henwood 2001). FTIR observed out thathigh huge peak at 3284 cm-1, 744 cm-1, 668 cm-1, 2925cm-1, 2853 cm-1, 1087 cm-1 and 1044 cm-1 showed the presence of alkyl halides, ethers, amine, carboxylic and esters with C-F, C-O, C-N and N-H stretch. Medium and coffee peaks including 3332cm-1, 2973cm-1, 1379cm-1 and 879cm-1 had been also determined in which the C2 trait of Centellaasiatica suggests same peaks as in C1 and the ethanol extract well-knownshows a few miscellaneous institution which include thiocarbonyl and phosphine with C=S, P-H and Si-oR bonds. Most of the peaks are much like the methonal extract of Centella species (Agme-Ghodke 2016). 601.26 can be attributed to alkyl halides, Alkyne with C-Br stretch, C-Cl stretch, C-H bend (Sugunabai 2015). Most of the peaks are just like the methonal extract of Centellaspecies (Agme-Ghodke 2016). The evaluation showed some of bonds which include aliphatic and fragrant chains and earrings (Byakodi 2018). The maximum in all likelihood compounds that may be expected to be gift within the extract exhibits anti-inflammatory houses, antimicrobial and anticancerous houses (Chandra 2007).The leaves of A. Lanata FTIR analysis results proved the presence of alcohols, phenols, alkanes, carboxylic acids, aldehydes, alkenes, nitro compounds, alcohols, carboxylic acids, esters, ethers, aliphatic amines and alkyl halides compounds (Mariswamy 2012). The FTIR spectroscopic research revealed the presences of alcohol, phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acid, aromatics, nitro compounds and amines had been discovered from ethanol leaf extract of Gmelinaasiatica via Florence and Jeeva (Florence 2015). The consequences of the prevailing have a look at are according with the test of (Florence 2015).

The end result of gift examine showed that, extracts of Centella asiatica leaves of each C1 and C2 consists of some vital compounds like Anthranilic acid, N-methyl-, butyl ester and

tert-Butyl(five-isopropyl-2-methylphenoxy)dimethylsilane with antimicrobial assets.Methonol extracts from the aerial elements of Centella asiatica resulted in the isolation of 10 Compounds with the aid of analyses of GCMS.Alkaloids are wealthy in remedy and represent maximum of the valuable tablets (Edeoga 2001). 1-Dodecene and 1-Hexadecene are olefins or alkenes which have antimicrobial homes which might be used in the production of detergents and biodegradable surfactants.Octadecane, three-ethyl-5-(2-ethylbutyl)- C26H54 has antioxidant, antifungal and anti inflammatory sports (Al-Marzouqi 2015). Tris[(trimethylsilyl)oxy]cholan-24-yl]-, methyl ester has antibacterial interest (Albin Jose 2015).Most of the organic actions of Centella asiatica are pentacyclic triterpene compounds in particular asiatic acid, madecassic acid and triterpene saponin-asiaticoside, madecassoside (Hashim 2011).The extraction of biologically energetic compound from plant fabric depending on the kind of the solvent used within the extraction observed out that the organic extracts furnished greater effective antimicrobial activity as compared to aqueous extracts.

Here, methanol and ethanol as incredible solvent for the extraction of antimicrobial compounds from flowers (Shan 2007, Sharma 2012). Our outcomes also verify this as ethanol extract suggests more effective compounds with bactericidal belongings and display maximum inhibition region 21mm toward E.Coli micro organism and 20mm closer to the fungus Aspergillus flavus and additionally display hobby in the direction of all of the microbes examined. Similar critiques enables our paintings are, antimicrobial functionality of C. Asiatica become showed that methanolic and ethanolic extract of C. Asiatica exhibited in opposition to all examined microorganisms (Arumugam 2011).(Zaidan 2005) said that, methanol extract of the leaves of C. Asiatica showed moderate hobby towards S. Aureus and penicillin resistant S. Aureus.Where,the aqueous and acetone extracts of leaf and callus were decided to be less powerful. The extracts of C. Asiaticaare effective to kill the micro organism B. Cereussurvive in excessive situations like excessive or low temperature(Utami 2011). The MIC of the extract become confirmed as zero.125µg/ml and DNA cleavage had been analyzed and ethanol extract indicates cell death with DNA cleavage and degradationfindings determined are in settlement with previous research of DNA cleavage.Antibiotic handled DNA cleaved and chromosome accrued display sizeable mobile lack of existence in recA mutans (Handa 2000).

Bacterial strains confirmed DNA degradation or cleavage after remedy with antibiotic (Bisset 2004).

The consequences of the present have a have a look at showed that, leaf extracts of both trait Centella asiatica (C1 and C2) ethanol extract personal bioactive compounds with antibacterial hobby towards many microorganism. When exceedingly studied, it's far located out that C1 shows higher interest to expand a novel sizeable spectrum of antimicrobial drug additives and may be used as antibacterial agent.

REFERENCES

1. Agme-Ghodke, V., Agmea, RN & Sagarb, AD (2016). "Analysis of bioactive compounds in leaves extract of Centella asiatica by using HRLC-MS & IR techniques " Journal of Chemical and Pharmaceutical Research**8**(8): 122-125.
2. Akinyem, K., Oluw, OK & Omomigbehin, EO (2006). "Antimicrobial Activity of Crude Extracts of Three Medicinal Plants Used in South-West Nigerian Folk Medicine on Some Food Borne Bacterial Pathogens." Afr. J. Trad. CAM**3**(4): 13–22.
3. Al-Marzouqi, A., Hameed, IH & Idan, SA (2015). "Analysis of bioactive chemical components of two medicinal plants (Coriandrum sativum and Melia azedarach) leaves using gas chromatography-mass spectrometry (GC-MS)." Afr. J. Biotechnol**14**(40): 2812-2830.
4. Albin Jose, J., Jasmine, E, Selvakumar, T & Srinivasakumar, KP (2015). "Bioactive compounds of Tinospora cordifolia by gas chromatography- mass spectrometry (GC-MS)." International Journal of Multidisciplinary Research and Development**2**(1): 88-97.
5. Alqahtani, A., Cho, JL, Wong, KH, Li, KM, Razmovski-Naumovski, V & Li, GQ (2017). "Differentiation of Three Centella Species in Australia as Inferred from Morphological Characteristics, ISSR Molecular Fingerprinting and Phytochemical Composition." Front Plant Sci**8**: 1980.
6. Arumugam, T., Ayyanar, M, Koil Pillai, YJ & Sekar, T (2011). "Phytochemical screening and antibacterial activity of leaf and callus extracts of Centella asiatica." Bangladesh Journal of Pharmacology**6**(1): 55–60.
7. Bisset, J., Doria, S, Nagra, N & Peck, V (2004). "Antibiotic-Induced DNA Cleavage: A Role of Restriction-Modification Systems " Journal of Experimental Microbiology and Immunology**5**: 101-105.
8. Byakodi, M., Bagewadi, Z & Muddapur, U (2018). "Phytoconstituents Profiling and Evaluation of Antimicrobial and Antioxidant Attributes of Methanolic Extract of Centella asiatica." Research Journal of Pharmaceutical, Biological and Chemical Sciences**9**(3): 493-500.
9. Chandra, M., Shio, A, Nariko, S, Yoshihiro, H & Biswanath, D (2007). "Flavonoids from the Stem-bark of Oroxylum indicum." Natural Product Sciences**13**(3): 190-194.
10. Cheng, C., Guo, JS, Luk, J & Koo, MW (2004). "The healing effect of Centella asiatica extract and asoaticoides on acetic acid induced gastric ulcers in rats." Life Science**74**: 2237-2249.
11. Chew, Y., Chan, EW, Tan, PL, Lim, YY, Stanslas, J & Goh, JK (2011). "Assessment of phytochemical content, polyphenolic composition, antioxidant and antibacterial activities of Leguminosae medicinal plants in Peninsular Malaysia." BMC Complement Altern Med**11**: 12.
Devkota, A. J., PK (2009). "Variation in Growth of Centella asiatica along Different Soil Composition." Botany Research Internationa**2**(1): 55-60.
12. Edeoga, H. E., DO (2001). "Alkaloid, Tannins and Saponin contents of some Nigerian medicinal plants." J. Med. Aromatic Plant Sci.**23**: 344-349.
13. Florence, A. J., S (2015). "FTIR and GC-MS spectral analysis of Gmelina asiatica L. Leaves." Science Research Reporter**5**(2): 125-136.
Gupta, Y., Veerendra Kumar, MH & Srivastava, AK (2003). "Effect of Centella asiatica on pentylenetetrazol induced kidling cognition and oxidative stress in rats." Pharmacol Biochem Behav**74**(3): 579-585.
14. Handa, N., Kusano, IK & Kobayashi, I (2000). "Cellular responses to postsegregational killing by restriction-modification genes." J. Bacteriol.**182**(8): 2218-2229.
15. Hashim, P., Sidek, H, Helan, MH, Sabery, A, Palanisamy, UD & Ilham, M (2011). "Triterpene composition and bioactivities of Centella asiatica." Molecules**16**(2): 1310-1322.
16. Hassan, M., Oyenwale, AO, Abduallahi, MS & Okonkwo, EM (2004). "Preliminary phytochemical and antibacterial investigation of crude extract of the root bark of Datarium microcarpum." J Chem Soc Nigeria**29**: 26-29.
17. Henwood, M. H., JM (2001). "Towards an understandingof the phylogenetic relationships of Australian Hydrocotyloideae (Apiaceae). ." Edinb. J. Bot.**58**: 269-289.
18. Lai, P. R., J (2004). "Antimicrobial and chemopreventive properties of herbs and spices. ." Current Medicinal Chemistry**11**: 1451-1460.
19. Lee, Y., Lee, DE, Lee, HS, Kim, KS, Lee, WS, Kim, SH & Kim, MW (2011). "Influence of auxins, cytokinins, and nitrogen on production of rutin from callus and adventitious roots of the white mulberry tree (Morus alba L.)." Plant Cell Tissue Organ Cult**105**(1): 9–19.
20. Mariswamy, Y., Gnanaraj, WE & Johnson, MAA (2012). "Ftir spectroscopic studies on Aerva lanata (L.) Juss. Ex Schult." Asian Journal of Pharmaceutical and Clinical Research**5**: 82-86.
21. Negi, P. (2012). "Plant extracts for the control of bacterial growth: efficacy, stability and safety issues for food application." International Journal of Food Microbiology**156**(1): 7–17.
22. Pittella, F., Dutra, RC, Junior, DD, Lopes, MTP & Barbosa, NR (2009). "Antioxidant and Cytotoxic Activities of Centella asiatica (L) Urb." International Journal of Molecular Science**10**(9): 3713-3721.
23. Senthil, J., Rameashkannan, MV & Mani, P (2016). "Phytochemical Profiling of Ethanolic Leaves Extract of Ipomoea sepiaria (Koenig Ex. Roxb) " International Journal of Innovative Research in Science, Engineering and Technology**5**(3): 3140-3147.
24. Shan, B., Cai, YZ, Brooks, JD & Corke, H (2007). "The in vitro antibacterial activity of dietary spice and medicinal herb extracts." International Journal of Food Microbiology**117**(1): 112-119.
25. Sharma, C., Aneja, KR, Kasera, R & Aneja, R (2012). "Antimicrobial potential of Terminalia chebula Retz. Fruit extracts against ear pathogens." World Journal of Otorhinolaryngology**2**: 8-13.
26. Sharma, D., Khosa, RL, Chansauria, JPN & Sahai, M (1996). "Antstress activity of Tinospora cordifolia and Centella asiatica extracts." Phytother Res**10**: 181-183.
27. Subathra, M., Shila, S, Devi, MA & Panneerselvam, C (2005). "Emerging role of Centella asiatica in improving age-related neurological antioxidant status." Exp Gerontol**40**(8-9): 707-715.
Sugunabai, J., Jeyaraj, M & Karpagam, T (2015). "Analysis of Functional Compounds and Antioxidant Activity of *Centella asiatica*." World Journal of Pharmacy and Pharmaceutical Sciences**4**(8): 1982-1993.
28. Tajkarimi, M., Ibrahim, SA & Cliver, DO (2010). "Antimicrobial herb and spice compounds in food." Food Control**21**(9): 1199-1218.
29. Uniyal, S., Singh, KN, Jamwal P & Lal, B (2006). "Traditional use of medicinal plants among the tribal communities of Chhota Bhangal." Western Himalayan Journal of Ethnobiology and Ethnomedicine**2**: 1-14.
30. Utami, C., Hatane, SE & Gorjian, M (2011). "The Application of three herbs; Chrysanthemum indicum, Centella asiatica and Andrographis paniculata to reduce bacteria in cow milk." International Journal of the Computer, the Internet and Management**19**(2): 38-43
31. Yoshida, M., Fuchigami, M, Nagao, T, Okabe, H, Matsunaga, K, Takata, J, Karube, Y, Tsuchihashi, R, Kinjo, J, Mihashi, K & Fujioka, T (2005). "Antiproliferative constituents from Umbelliferae plants VII. Active triterpenes and rosmarinic acid from Centella asiatica." Biol Pharm Bull**28**(1): 173-175.
32. Zaidan, M., Noor Rain, A, Badrul, AR, Adlin, A, Norazah, A & Zakiah, I (2005). "In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method." Trop Biomed**22**(2): 165-170.