ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Antimicrobial, Anti-Inflammatory Effects Of *Centella asiatica* Leaf And Their Cytotoxic Effects On MCF-7 And HeLa Cell Lines.

Yoshini Kathleen Vasagam, and Shamala Marimuthu*.

Department of Biotechnology, Manipal International University, 71800 Nilai, Negeri Sembilan Darul Khusus, Malaysia.

ABSTRACT

Centella asiatica leaf contains a vast range of phytochemicals which proven with its medicinal properties. In present study, ethanol extract of *Centella asiatica* was studied for its phytochemical and pharmacological properties. *C. asiatica* extracts indicates the presence of Asiatic acid, Asiaticoside, Madecassoside and Madecassic acid. The extract containing these valuable compounds were tested against microorganisms using a disc diffusion method. It was observed that the extract showed greater bactericidal on gram negative bacteria compared with gram positive bacteria and there was antifungal activity towards *Aspergillus niger.* For anti-inflammatory test, the highest concentration of extract (300mg/ml) gave the highest denaturation percentage of 51%. The MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium assay was used to detect the viability of the cells when tested with *Centella asiatica* leaf extract at various concentration based on the formazan production from the bromide. Besides that, 200µl *Centella asiatica* leaf extract with a concentration of 100mg/ml exhibited anticancer activity on MCF-7 and HeLa cells. The findings from this study indicated that *Centella asiatica* leaf extract can be used in the pharmaceutical industry as an antimicrobial, anti-inflammatory and anticancer drug.

Keywords: *Centella asiatica*, Antimicrobial, Anti-Inflammatory, Cytotoxicity

https://doi.org/10.33887/rjpbcs/2020.11.1.1

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Centella asiatica, a pantropical herb commonly known as pegaga in Malaysia and its utilised to treat a vast range of indications. It is exposed to an extensive phytochemical, analysis and clinical examinations [20]. The potential of *Centella asiatica* isn't just socially desirable, however is economically reasonable, manageable and involves minimal risks with low toxicity. Belonging to the *Apiaceae* family, its natives are tropical countries which explains the creeper herbaceous plants perennial properties. It was used as herbal remedy in Ayurveda and traditional Chinese medicine where it was used to treat various chronic diseases for centuries.

Ethanol extract of *Centella asiatica* had stimulated cell-mediated immune system by increasing white blood cell phagocytic function. This has been a large help in discriminating the cancer cells from the body [12].

Centella asiatica also shows bactericide activity against wide range of microorganism & enteric pathogens. It's antimicrobial properties, disrupts the microorganisms' genetic material to inhibit growth and kill it [13]. Also, it increases the collagen levels to repair the microbial infected dermis.

Centella asiatica provides protection against diseases by enhancing immunity & reducing inflammation in the body [21]. Anti-inflammatory properties stimulate collagen synthesis and protein denaturation, where it accelerates cicatrisation and grafting of wounds. Additionally, it is also promotes fibroblasts proliferation and extracellular matrix synthesis in wound healing.

Lately due to lifestyle and habitual changes, people of all ages are prone to life threatening diseases. Modern medicines cost a fortune and consist of variety of side effects upon its usage. This has given a lot of attention towards herbal medicine where C*entella asiatica's* properties, medicinal and nutritional value were determined for pharmaceutical usage [7].

METHOD AND MATERIALS

Collection and extraction of *Centella asiatica* leaves

Fresh fully matured *C. asiatica* leaf was washed thoroughly under running tap water followed by sterile water and dried under the oven at the temperature of 30°C for 7 days. Then, the dried samples were homogenized to fine powder form. The dry weight and the moisture content were calculated [11].

$$MC = [(w-d) \div w] \times 100$$, Where, 'w' is wet weight and 'd' is dry weight.

20g of the powered leaf samples were macerated with 250ml of absolute ethanol and incubated at 37°C in a shaker incubator with 150rpm for four days [15]. The extracts were then filtered, and solvent was evaporated using rotary evaporator at 40°C.

Thin Layer Chromatography (TLC) Analysis

The prepared plant extracts were applied on pre-coated TLC plates using capillary tubes and developed in a TLC chamber using 3ml of ethyl acetate with 27ml of hexane as ratio of 1: 9 as mobile phase [19]. The developed TLC plates were air dried and observed. The movement of the analyse was expressed by its retention factor (Rf). The values were calculated based on formula below:

Rf = Distance travel by solute / Distance travel by solvent

Antimicrobial Analysis

The plant extract concentration of 300 mg/ml was tested for antimicrobial activity using disc diffusion assay [16]. The test microorganisms used in this study are bacteria: (Gram positive - *Staphylococcus aureus*, *Bacillus cereus*, and Gram negative - *Pseudomonas aeruginosa*, *Escherichia coli*) and fungal strain *Aspergillus niger* were used. Bacteria plates was incubated at 37°C for 24 hours and zone of inhibition was measured. As for positive control, the antibiotic Ciprofloxacin (10µg/ disc) was used against the microorganisms.

Cytotoxicity Analysis (MTT Assay)

MCF-7 cells (human breast carcinoma cells) and HeLa cells (cervical cancer cells) were tested [6]. Each well was filled with100µl of cells and incubated at 37°C for 24 hours. Then, *Centella asiatica* extract at concentration of 100mg/mL were added at volumes of 50µl, 100µl and 200µl into the wells and incubated for 72 hours at 37°C. Then, 10µl of MTT assay with 100 µl of fresh medium was pipetted into the cells and the plate was incubated for 4 h at 37˚C in the dark [9]. Then, 300µl SDS-DMSO solution was added to the wells and incubated again for another 4 h. Within an hour, 100µl of 0.04N HCl-Isopropanol was added into the wells. The absorbance of coloured product formed was finally measured at 570 nm using an ELIZA reader with the value is proportional to the number of viable cells in the test sample [1]. Cell viability for each volume of extract was expressed as percentage relative to the untreated cells, which was calculated using the formula:

Cell viability (%) = (Average absorbance of treated cells / Average absorbance of untreated cells) ×100

Anti-inflammatory analysis

Anti-inflammatory analysis was done with minor modifications [8]. The reaction mixture was consisting of test extract at different concentrations 0.05ml of 100mg/ml, 0.05ml of 200mg/ml and 0.05ml of 300mg/ml and 0.45ml of BSA aqueous solution of bovine albumin fraction. 0.05ml of DMSO was used as positive control and 0.05ml of distilled water was used as negative control.

The samples were incubated at 37^{o}C for 20 min and then heated at 57^{o}C for 20 min. After cooling the samples, the turbidity was measured spectrophotometrically at 660 nm. The experiment was performed in triplicate. The percentage of protein denaturation was calculated using the below formula:

Percentage inhibition = (Abs control – Abs sample) X 100/ Abs control

RESULTS AND DISCUSSION

Extraction and TLC Analysis

The dry weight obtained was 75.9g, and moisture content was 87.6%. *Centella asiatica* leaf powder was macerated with absolute ethanol. Maceration breaks down and softens the sample making it easy to release the wanted compounds and ethanol is a highly polar solvent [3].

Based on Table 1, the compounds Asiatic acid, Asiaticoside, Madecassoside and Madecassic acid were identified.

Table 1: Retention factor and colour of each compound

Compound	Colour	Retention Factor
Asiatic Acid	**Dark Blue Green**	**0.18 ± 0.2**
Asiaticoside	**Light Orange**	**0.90 ± 0.4**
Madecassoside	**Dark Orange**	**0.95 ± 0.5**
Madecassic Acid	**Dark Yellow**	**0.98 ± 0.8**

Antimicrobial Analysis

Based on Table 2, it shows that the *Centella asiatica* leaves' extract has a strong antimicrobial effect on tested microorganisms. Gram negative bacteria had the widest clear zone due to highest the cellular disruption. Followed by gram positive bacteria having an average clear zone and fungal strain with the smallest clear zone. Asiaticoside and Madecassoside compounds had the tendency to stop the bacterial strains from proliferation by disrupting its DNA leading to death which lead to a formation of a clear zone. However, Asiatic acid contained fungal spores from sporulating allowing only a minor inhibition of growth [4]. The compounds from *Centella asiatica* leaves' extract, not only works with healing bacterial and fungal infections on skin but also increases the collagen levels to repair the microbial infected dermis [10].

ISSN: 0975-8585

Table 2: Antimicrobial properties against the microbial strains

Microorganisms	Ciprofloxacin (10µg/disc)	*Centella asiatica* extract (300mg/mL)
B.cereus	26 ± 0.9	17 ± 1.2
S.aureus	36 ± 0.7	11 ± 0.9
E.coli	25 ± 0.2	27 ± 0.5
P.aeruginosa	33 ± 0.5	14 ± 0.8
A.niger	0 ± 0	10 ± 0.7

Anti-inflammatory analysis

Anti-inflammatory activity is tested on BSA using *Centella asiatica* leaves' extract. Based on Table 3, *Centella asiatica* leaves' extract inhibited the protein denaturation in a concentration dependent where higher the extract concentration the higher the protein denaturation. This is due to the compound Asiatic acid which works well in healing inflammation, swelling and memory related. Madecassic acid plays a role in promoting cell proliferation and reduction of dead cells also accumulation of debrises [17]. The presents of both of these compounds in the test sample shows a strong properties in reducing protein denaturation and improving the cells fibroblastic density [18]. The extract increases cellular proliferation and collagen synthesis at the wound site, as evidenced by increase in DNA, protein and collagen content of granulation tissues.

Table 3: Anti-inflammatory analysis of *Centella asiatica* leaf extract against bovine serum albumin based on protein denaturation percentage.

Sample	Constituents with BSA	Percentage of protein denaturation (%)
T1	100mg/ml *C.asiatica* extract	18 ± 0.6
T2	200mg/ml *C.asiatica* extract	39 ± 0.7
T3	300mg/ml *C.asiatica* extract	51 ± 0.4
C+	DMSO	73 ± 0.8
C-	Distilled water	5 ± 0.5

Cytotoxicity Analysis (MTT Assay)

In both MCF-7 and HeLa cell lines, it was evident that the higher volume of *Centella asiatica* leaves extract caused major cytotoxicity effects to the cells. High volumes of *Centella asiatica* leaves' extract used, increased the cell apoptosis [5]. Thus, causing high levels of anti-tumour properties. As per the analysis, *Centella asiatica* leaves' extract causes antitumor activity by direct inhibition of DNA synthesis which stops the tumour proliferation and causes apoptosis [2]. The Asiaticoside and Madecassoside compounds are known to be defectors of tumour causing cells [14]. It detects the malfunctioning or over dividing cells and uses it as a target to detect its genetic material. Then, it inhibits and slowly stops the DNA synthesis which causes the cells to become memoryless, energy-less and helpless. When the cells have no powers, it cannot proliferate leading to cell death, apoptosis.

CONCLUSION

In conclusion, present study revealed that compounds like Asiatic acid, Asiaticoside, Madecassoside and Madecassic acid obtained from ethanolic extract of *Centella asiatica* leaves. These compounds have great antimicrobial, anti-inflammatory and cytotoxicity properties. For antimicrobial activity, the extract on gram negative bacteria showed most inhibition of bacterial growth, followed by gram positive bacterial and least inhibition by the fungal strain. Secondly, increasing concentrations of the extract gave increasing percentage of protein denaturation showing anti-inflammatory activity.

ISSN: 0975-8585

REFERENCES

[1] Babykutty S, Padikkala J, Sathiadevan P, Vijayakurup V, Karedath T, Azis A, Srinivas P, Gopala S. African Journal of Traditional, Complementary and Alternative Medicines 2009; 6 (1): 9 – 16.

[2] Bahuguna A, Khan I, Vivek K, Sun CK. Bangladesh J Pharmacol 2017; 12:115-118.

[3] Coskun O. Northern Clinics of Istanbul 2016; 3(2): 156-160

[4] Dash BK, Faruquee HM, Biswas SK, Alam MK, Sisir SM, Prodhan UK. Life Sciences And Medicine Research 2011; 5: 1-5.

[5] Frederico P, Rafael C, Dutra E, Dalton D, Miriam TP, Barbosa NR. International Journal of Molecular Sciences 2009; 10: 3713-3721.

[6] Graidist P, Martla M, Sukpondma Y. Nutrients 2015; 7: 2707-2718.

[7] Gohil J, Patel A, Gajjar K. Indian Journal of Pharmaceutical Sciences 2010; 72(5): 546-556.

[8] Govindappa M, Sadananda T, Channabasav R, Vinay B. International Journal of Pharma and Bio Sciences 2011; 2(2): 275-285.

[9] Hanisa H, Mohdazmi M, Suhaila M, Hakim MN. International Journal Of Pharmacy And Pharmaceutical Sciences 2014; 6(1): 388-392.

[10] Hossain MS, Tuly FA, Akter S, Arman MSI, Aziz MA, Mahmud ME, Das P, Babu MH, Islam MM. Plant Science Publishing Group 2018; 6(1): 1-7.

[11] Huie, CW. Analytical and Bioanalytical Chemistry 2002; 37(3): 23-30

[12] Jambunathan S, Bangarusamy D, Raghunathan P, Sundaravadivelu S. Asian Pacific Journal of Tropical Medicine 2014; 7(1): 405-409

[13] Lalitha V, Kiran B, Raveesha KA. World Journal Of Pharmacy And Pharmaceutical Sciences 2013; 2(6): 4744-4752.

[14] Mutua PM, Gicheru MM, Makanya AN, Kiama SG. International Journal of Morphol 2013; 31(4):1322-1327.

[15] Rattanakom S, Yasurin P. Oriental Journal Of Chemistry 2015; 31(4): 2453-2459.

[16] Rishikesh M, Rahman MD, Siddiqul SM, Rahman M. International Journal Of Pharmaceutical Science And Research 2012; 3(9): 3323-3330.

[17] Sakat S, Juvekar R, Gambhire N. International Journal of Pharmacy and Pharmaceutical Sciences 2010; 2(1):146-55.

[18] Seevaratnam V, Banumathi P, Premalatha MR, Sundaram SP, Arumugam T. International Journal of Pharmacy and Pharmaceutical Sciences 2012; 4(5): 0975-1491

[19] Sherma J, Fried B. Hand Book of Thin Layer Chromotography (3rd Edition). Marcel Dekker, Inc., New York, 2003, pp.1048

[20] Singh S, Gautam A, Sharma A, Batra A. International journal of pharmaceutical sciences review and research 2010; 4(2): 9-17.

[21] Sushen U, Chouhan A, Ali K, Ranjesh V. European Journal Of Pharmaceutical 2017; 4(9): 261-268.