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<Review>

The Potential of *Centella asiatica* (Linn.) Urban as an Anti-Microbial and Immunomodulator Agent: A Review

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Abstract – *Centella asiatica* (Linn.) Urban (Umbelliferae) which is also known as 'pegaga' is highly consumed and eaten raw as 'ulam' in Malaysia. *C. asiatica* is used in traditional medicines to treat various health conditions such as rheumatism, inflammation, syphilis, skin diseases and diarrhoea. Various reports exhibited that the crude extracts and isolated bioactive compounds of *C. asiatica* possessed a broad range of pharmacological activities such as anti-oxidant, anti-diabetic, anti-tumor, wound healing, anti-microbial, anti-inflammatory, immunomodulatory, hepatoprotective and memory enhancing properties. The pharmacological validation on anti-microbial and immunomodulatory of *C. asiatica* is very limited and several existence review papers related for this plant had not been focused for both activities. This review therefore attempts to combine the existing literature to offer immense scope for researchers engaged in validation of the traditional claims and bioactivities of this plant related with anti-microbial and immunomodulatory potential.
Keywords – Anti-microbial, *Centella asiatica* (Linn.) Urban, immunomodulatory, pegaga

Introduction

Recently, infectious diseases have highest concern for healthcare providing organizations, mainly in developing countries. 'Emerging infectious diseases' (EIDs) is defined as a cluster of diseases which have occurred previously or first time introduced in a population that is rapidly increasing in prevalence or geographic range.[1] The study by Church (2004) had been shown that the causes of EIDs were highly associated with changes in human behavior, global population and environmental. There are majorly managed by preventive measures including vaccination programs and using suitable antibiotics to treat existence diseases.[2] However, antibiotic has many serious adverse effects like bone marrow suppressing effects, drug resistances, tolerance, allergic reactions and removing normal flora bacteria in body. In addition, it causes tissue damage due to extended consumption which produces toxic reactive oxygen species and also interfered with the normal body's immune system and reduced immune response towards specific antigen.[3] Thus, it is better to provide other alternative from natural products that acts as anti-microbial or strengthen the normal body's immune defense system for managing microbial infection instead of using modern synthetic agents.

The immune system is important to human health as it functions to protect body from invading pathogens and treat the existence of diseases.[4] Even a minor infection will produce seriously bad effects when the host immune system is defected. Instead of that, the progression of abnormal condition such a cancer is believed to initiate by alteration of host's immune system. One of the ways in reducing this problem is to modify the host's immune responses by increasing the capability of this system in preventing or eliminating the etiologic agents that caused diseases.[5] The use of a variety of agents is aimed to modulate immunological parameters which includes non-specific host defenses, humoral antibody or cell-mediated immune responses that required to control numerous of diseases.[6] The modification of immune responses that function to up and down the immune alertness to treat diseases is known as immunomodulation.[7] The use of immunomodulators for either as a prevention or treatment of various diseases that related with defective immune

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responses became the focus of major attention since a long times ago.[8] The main objectives of immunomodulators are either to enhance immune responses that applied in infectious and immunodeficiency diseases or to suppress them as a treatment for inflammation and autoimmune diseases.[9] Besides, the use of immunostimulant also important as an adjuvant to chemotherapy for various diseases.[10]

Natural products which originated from plants, fungal, bacterial and marine animal sources offer a large cluster of various chemical entities with a numerous of pharmacological activities and lesser number of side effects against synthetic medicinal agents. In 2013, US Food and Drug Administration approved approximately 1453 new chemical entities that have been isolated from natural products.[11] One of the well-known natural products belongs to the group of medicinal plants and herbs which are rich in bioactive compounds and beneficial for human health. There're a great interest of scientists into these groups of natural products, because of the modern therapeutic medicines that are associated with adverse side effects and are costly to purchase in comparison with herbal products. They also have been widely used as medications, nutritive products, and health supplements since prehistoric times.[12] Many such preparations from medicinal plants have been reported as a medicines to improve body immune system include *Centella asiatica*, *Glycyrrhiza glabra*, and *Allium sativum*.[13] Herbs that are high content in flavonoids, triterpene glycosides, vitamin C or carotenoids have a potential to enhance immune functions.[13,14]

Centella asiatica (Linn.) Urban is derived from family Umbelliferae. The taxonomy of *C. asiatica* is listed in Table 1. Their derivatives have been used as a traditional herbal medicine in Malaysia and other parts of Asia for hundreds of years. This plant is traditionally used for antimicrobial, immune booster, wound healing and treatment of skin-related inflammatory conditions such as eczema and psoriasis.[15,16] Nowadays, *C. asiatica* has attracted significant research and commercial interests due to its health-promoting bioactive compounds.[17] Based on the previous studies, the biological activities of *C. asiatica* have been accounted due to the presence of pentacyclic triterpene acids in the leaves (asiatic acid and madecassic acid) and their respective glycosides (asiaticoside and madecassoside). Almost of the phytochemical studies were concentrated on leaves and the components are differ depending upon the geographical distribution.[18] Under this background the present work was carried out to review the anti-microbial and immunomodulatory activities of extract and bioactive compounds derived from *C. asiatica*.

Table 1. Taxonomy of *Centella asiatica*

Classification	Name
Kingdom	Eukaryota/Plantae
Subkingdom	Embryophyta
Division	Spermatophyta
Subdivision	Angiospermae
Class	Dicotyledoneae
Subclass	Rosidae
Superorder	Aralianae
Order	Apiales/Umbelliflorae
Family	Apiaceae/Umbelliferae
Subfamily	Mackinlayoideae/Hydrocotyle
Genus	Centella
Species	Asiatica

Table 2. Vernacular (Dialect) names of *Centella asiatica*

Language/region	Vernacular (Dialect) name
Bengali	Thankuni
China	Fo-ti-tieng, Chi-hsueuh-ts'ao
Hawaii	Pohe Kula
Hindi	Mandookaparni
Malay/Malaysia	Pegaga
Malayalam	Kodagam
Nepal	Ghodtapre
Urdu	Brahmi
USA	Indian Pennywort, Marsh Pennywort

Plant properties

C. asiatica is a creeping, perennial and slender trailing herb. It has reddish prostrate stolons and long rooting at the nodes. Its' glabrous leaves with scalloped edges have reniform, entire, crenate which about 1.3 cm to 7 cm in diameter.[19] Flowers are white or light purple-to-pink in color which are covered by bracts. These flowers in quantity of 3 to 4 are clustered in an umbel.[18] The small and compressed fruits contained 7 to 9 ridged and have sized of 8 mm long. The mericarps are rounded at the top and curved meanwhile the lateral seeds are compressed.[20]

Botanical synonyms and vernacular names

Centella asiatica (Linn.) Urban also has other botanical synonyms as *Centella coriacea* Nannfd., *Hydrocotyle asiatica* L., *Hydrocotyle lunata* Lam., *and Trisan- thus cochinchinensis* Lour.[21] Among communities, this plant is famously known as "Pegaga," in Malaysia,[22] "'Daun Kaki Kuda," "Antanan gede," and "Pegagan" in Indonesia,[15] "Mandookaparni," "Thankuni," "Manimunni" and "Valleri"

in India,[23] "Luei Gong Gen" or "Tung Chain" in China,[24] "Indian Pennywort" and "Marsh Pennywort" in USA,[19] and "Bua-bok" in Thailand.[25] Some vernacular names of this plant are summarized in Table 2.

Distribution

C. asiatica is found almost all over the world. It is a native plant to warmer region countries of both North and Sourth hemispheres including Africa, Asia, India, southern United States of America, Central America and South America, Australia, Venezuela and Madagascar.[26] The plant grows most abundant during rainy season mainly in marshy and dampy areas.[23]

Traditional uses

Dried whole plant, leaves and stems are the most used part of *C. asiatica* for remedial purposes.[18] In Malaysia and Indonesia, it is highly consumed and eaten raw as a salad or in different formulas including capsule, pill, tea and juice. Sometimes, the Malaysian peoples prepared and served it with sweet potato and coconut milk to reduce its' bitterness and the fresh leaf also is eaten to increase lactation after birth.[27,28] In numerous region of the world, *C. asiatica* is used to increase health. Chinese and Thai people always used this plant as a drinking water for thirst reducing purpose and as a cooling agent.[15] In Southeast countries in Asia, it is traditionally used for the treatment of variety of illnesses such as rheumatism, epilepsy, hysteria, dehydration, inflammation, syphilis, mental illness, skin diseases and diarrhoea.[16]

Centella asiatica: Anti-microbial activities

Antibiotic resistant has been a great concern of health problem throughout the world. During the last decades, development of drug resistance has significantly increased due to the widespread usage of antibiotics. On the other hand, the resistant may be also due to the ability of bacteria to quickly adapt to their environmental changes. As a result, this incidence has led to emergence of various illnesses and it is important to find a new anti-bacterial agent from other alternative sources. Recently, natural products have been important source of medicinal properties in the scope of anti-microbial. Currently, medicinal plants are still used by up to 80% of population worldwide particularly in developing countries for the treatment and prevention of diseases. *C. asiatica* is one of the essential medicinal plants which commonly used in traditionally for the treatment of several ailments including infectious diseases. Therefore, to support the statement, the anti-microbial activities of *C. asiatica* against various human pathogens are emphasized in Table 3.

Preliminary study using disc diffusion method presented that the ethanol extracts of *C. asiatica* had anti-bacterial activities against human pathogenic bacteria such as *Proteus vulgaris*, *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*. In this research, ethanol extract of *C. asiatica* exhibited anti-bacterial activities with average of inhibition zone were 16 to 19 mm.[29] Moreover, the similar extract of *C. asiatica* has been reported to possess broad inhibition of spectrum activities against *Shigella species, Vibrio cholera, and S. aureus*. Punch well and agar dilution methods were used to determine this anti-bacterial activities and the result showed that the ethanol extract at a concentration of 400 mg/mL inhibited most significant inhibitory properties against those bacteria.[30] The ethanol extract of *C. asiatica* was also effective against the Gram-negative bacteria. On the basis of disc diffusion method, four different concentrations (100, 200, 300 and 400 μg/disc) were used to observe anti-bacterial activity of *C. asiatica*. According to this study, mild anti-bacterial activity was also noted against *Pseudomonas aeruginosa* with average inhibition zone was 6 mm, compared to the standard drug.[31] Susceptibility test of anti-bacterial agent is performed by determining its minimum inhibitory concentration (MIC) using broth micro-dilution and followed by quantifying a minimal bactericidal concentration (MBC). Using a modified resazurin microtiter plate to determine MIC value and continued by quantifying MBC by mannitol salt agar, the other research reported that the ethanol extract of *C. asiatica* had strong anti-bacterial activity against isolated *S. aureus* from milk samples of dairy cows with MIC and MBC values of 8 and 16 mg/mL respectively.[32] It has been known, under certain environment, bacteria have their defensive mechanism which allows them to survive. Based on the previous study which using two types of bacteria that able to survive in high osmotic stress such as *Bacillus cereus* and *Listeria monocytogenes*, the ethanol extract of *C. asiatica* showed strong anti-bacterial activities with MIC values of 16 and 8 μg/mL, and MBC values of 16 and more than 32 μg/mL independently.[33] Furthermore, *C. asiatica* also demonstrated its activity against resistant bacteria. Zaidan and team reported that *C. asiatica* showed anti-bacterial activity against Gram-positive and Gram-negative bacteria by standard disc diffusion method. Furthermore, using MIC to verify its susceptibility, 1 000 μg/disc of methanol extract of *C.*

Table 3. Anti-microbial activities of *Centella asiatica*

No.	Tested substances	Anti microbial properties	Method	Tested microorganisms	Results	References
1.	Ethanol extract	Anti-bacterial	Disc diffusion	*P. vulgaris*, *S.aureus*, *B.subtilis* and *E.coli*.	Range of inhibition zone were 16-19 mm	29
2.	Ethanol extract	Anti-bacterial	Agar dilution	*Shigella sp., V. cholera* and *S.aureus*	400 mg/mL of agar	30
3.	Ethanol extract	Anti-bacterial	Disc diffusion	*P. aeruginosa*	Average inhibition zone was 6 mm	31
4.	Ethanol extract	Anti-bacterial	Resazurin microtiter plate	*S. aureus*	MIC and MBC values of 8 and 16 mg/mL	32
5.	Ethanol extract	Anti-bacterial	Broth microdilution	*B. cereus* and *L.monocytogenes*	MIC values of 16 and 8 µg/mL, and MBC values of 16 and >32 µg/mL	33
6.	Methanol extract	Anti-bacterial	Disc diffusion	*S. aureus* and MRSA	1 000 µg/disc of methanol extract of *C. asiatica* was effective to inhibit the growth of tested bacteria	34
7.	Rich flavonoid extract	Quorum sensing and virulence factors inhibiton	Violacein, pyoyanin production, swarming motility, and antibiofilm production	*C. violaceum* and *P. aeruginosa*	The extract at 300 µg/disc completely inhibited violacein production. The extract at 400 µg/mL completely inhibited pyocyanin production, biofilm production as well as elastolytic and proteolytic activity in *P. aeruginosa* PAO1	35
8.	Methanol extract	Anti-fungal	Spore germination assay	*F. udum, D. monoceras*	The result showed that the extract at 5000 µg/mL completely inhibited the spore germination of *F. udum*, *D. monoceras*	36
9.	Ethanol extract	Anti-fungal	Disc diffusion	*A. niger* and *C. albicans*	Inhibiton zone was 16 mm against *A.niger* and 15 mm agaisnt *C. albicans*	29
10.	Aquoes extract	Anti-fungal	Microdilution method	*C. cladosporidies, A. flavus*, and *F. oxysporum*	Aqueous extract of *C. asiatica* had satisfied activity in inhibiting those that of fungi with MICs in the range of 80 to 90%.	37
11.	Silver nanoparticle synthesised using C. asiatica	Anti-bacterial	Disc diffusion	*S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa*	AgNPs has strong anti-bacterial activity against *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa* with inhibition zone of 21.3, 19.4, 16.2 and 18.8 mm, respectively	40
12.	Asiatic acid	Anti-bacterial	Broth microdilution	*E. faecalis* and *E. coli*	Against *E. faecalis*, MIC and MBC values of 20 and 32 µg/mL and against *E. coli*, MIC and MBC value of 24 and 36 µg/mL.	42
13.	Ursolic acid	Quorum sensing and virulence factors inhibiton	Virulence factor inhibiiton methods	*E. coli*	Ursolic acid at 50 µg/mL completely inhibited virulence factors of *E. coli*, including P-fimbriae, curli fibres and alpha-hemolysin	43
14.	Volatile oil	Anti-bacterial	Microplate dilution method	*B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa* and *S. sonnei*	MIC values of in the range of 0.039 to 1.29 µg/mL against tested bacteria	44

asiatica was effective to inhibit the growth of *S. aureus* and methicillin resistant *S. aureus*.[34]

Quorum sensing (QS) is known to play an important role for the defense mechanism of bacteria towards antibiotics. QS is a phenomenon in which bacteria able to converse via secreted signaling molecules called auto-inducers, which regulate the expression of particular genes. This process is involved in controlling gene expression responsible for bacterial physiological function including biofilm formation as well as virulence factor production. Thus, disruption of QS has been proposed as a new anti-infective strategy. It has been reported that rich flavonoid extract of *C. asiatica* showed its ability in disruption of QS in *P. aeruginosa*. The extract was able to inhibit violacein production without affecting bacterial growth. The ethanol extract of *C. asiatica* exhibited anti-QS in *Chromobacterium violaceum* CV026. Briefly, the extract at 80 μg/disc demonstrated violacein inhibition with the 22 mm pigmentless zone. *C. asiatica* was also able to inhibit QS-regulated production of violacein production in *C. violaceum* CV026. The extract at concentration of 300 μg/disc inhibited violacein production completely. Further experiment was performed to determine the effect of *C. asiatica* on *N*-acyl homoserine lactones (AHL) in *C. violaceum*. The outcome revealed that the ethanol extract of *C. asiatica* (100 - 400 μg/mL) enabled to inhibit AHL-mediated violacein production in a concentration-dependent manner. This extract also demonstrated inhibition of QS phenotypes including pyocyanin production as well as biofilm formation of *Pseudomonas aeruginosa* in a similar pattern. The extract at 400 μg/mL completely inhibited pyocyanin production, biofilm production as well as elastolytic and proteolytic activity in *P. aeruginosa* PAO1. Moreover, the ethanol extract of *C. asiatica* (50 μg/mL) also inhibited swarming motility of *P. aeruginosa* PAO1. These findings indicated that *C. asiatica* is not only directly active towards suspected bacteria, but also effective in disrupting mechanism involved in bacteria defensive system, including QS.[35]

Superficial fungal infection has also contributed in increasing public health problem. The commensal microbes, including *Candida albicans* able to cause systemic infection in human. The incidence of candida infection has significantly increased mainly in immunocompromised patients. According to a previous work, anti-fungal activity of *C. asiatica* has also been reported. Study by Singh and team using spore germination assay, six different concentrations (1000 - 10000 μg/mL) were used to determine the anti-fungal activity of the methanol extract of *C. asiatica*. The findings showed that the extract at 5000 μg/mL completely inhibited the spore germination of *Fusarium udum*, *Dreschslera monoceras.* Besides, this extract significantly enabled to inhibit the growth of tested fungi such as *F. udum*, *D. monoceras*, *D. turtica* and *D. oryzae*. Additionally, the synergistic effect also detected when the extract combined with *Andrographis paniculata*.[36] Dash and team who used disc diffusion method, presented strong anti-fungal activity against *Aspergillus niger* with the inhibition zone were 16 and 14 mm respectively after treated with ethanol and petroleum ether extract. In case of *C. albicans*, the ethanol and chloroform extracts demonstrated strong activity with inhibition zone of 15 mm. All these results assumed that the ethanol extract had strong anti-fungal activity against *A. niger* and *C. albicans* which are comparable to ketocanozole as a positive control.[29] Separately, screening of anti-fungal activity against some important seed borne fungi including *Cladosporium cladosporidies, Aspergillus flavus*, *Penicillium* sp. and *Fusarium oxysporum* recorded that aqueous extract of *C. asiatica* had satisfied activity in inhibiting those fungi with MICs in the range of 80 to 90%.[37] *C. asiatica* herb is one of recommended plant that useful for the treatment of dermatological disease. This plant also has important activity in facilitating wound healing process *in vitro* and *in vivo*.[38,39] Recently, nanoparticle has attracted interest of many researcher due to its promising area for development of pharmaceutical product. Various techniques such as physical and chemical have been proposed to synthesize silver nanoparticle. However, the use of chemical as a reducing and stabilizing agents may lead to increase of toxic and hazard in environment. Green synthesis of silver nanoparticles using natural products has provided outstanding prospect to reduce the use of chemical agents. In addition, green synthesis using plant as reducing agents also provides increasing its pharmacological activities due to the synergistic interaction between natural product and silver itself. According to the previous work, silver nanoparticles (AgNPs) had been successfully synthesized using callus of *C. asiatica*. Further study found that synthesized AgNPs had a strong anti-bacterial activity which was comparable to the positive control. After 24 hours incubation period, the inhibition zone was appeared on a sterile disc paper impregnated with silver nanoparticle. The results showed that AgNPs had strong anti-bacterial activity against *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa* with inhibition zone of 21.3, 19.4, 16.2 and 18.8 mm, respectively.[40] Another study also recorded that synthesized AgNPs trough green synthesis using *C. asiatica* extract performed an anti-bacterial activity against *S. aureus*. AgNPs exhibited

anti-bacterial activity against tested bacteria with MIC value of 7.4 lg/mL.[41] These results indicated that *C. asiatica* along with other substance as well as the plants itself have a potent anti-bacterial activity against various human pathogens.

Moreover, *C. asiatica* contained numerous of secondary metabolic compounds which have various pharmacological properties including anti-microbial. Asiatic acid is one of a major phytochemical compound contained in the plants. Study by Liu and team illustrated that asiatic acid had satisfactory broad-spectrum anti-bacterial activity against Gram-positive and Gram-negative bacteria. It was found that asiatic acid showed strong anti-bacterial activity against *Enterococcus faecalis*, a Gram-positive bacteria and *E. coli*, Gram-negative bacteria with MIC and MBC values of 20 and 32 μg/mL and 24 and 36 μg/mL, respectively. Further investigation was performed to confirm the anti-bacterial action of this compound and the results showed that treated bacteria with asiatic acid at concentration of two-fold higher than its MIC value lead to alter membrane integrity. In addition, this similar dosage increased the release of potassium ions as well as protein in treated bacteria.[42] Besides, asiatic acid and ursolic acid from *C asiatica* had a potent anti-bacterial activity against clinical uropathogenic *E. coli*. These compounds showed the anti-bacterial activity with MIC values in the range of 512 to 1024 μg/mL. Moreover, both of these compounds were able to reduce production of virulence factor. Asiatic acid and ursolic acid at 50 μg/mL inhibited virulence factors of *E. coli*, including P-fimbriae, curli fibres and alpha-hemolysin. From this study, reduction of bacterial motility was also recorded.[43] Furthermore, *C. asiatica* are also important source of essential oil. Oyedeji and Afolayan have isolated volatile oil from this plant and further conducted the anti-bacterial properties testing using microplate dilution method. The findings showed that essential oil from *C. asiatica* exhibited strong anti-bacterial activity against wide range of bacterial, including *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa* and *S. sonnei* with MIC values in the range of 0.039 to 1.25 μg/mL. These results illustrated the broad spectrum anti-bacterial effects of essential oil from *C. asiatica*.[44]

Centella asiatica: Immunomodulatory activities

Immunomodulator is a constituent which able to enhance or regulates the immune system comprising both innate and adaptive immune responses. Recently, various medicinal plant products have become a subject for scientific investigation to modulate immune system. The immune system is a basic defense system of the body against foreign substances and pathogens. Its purpose is to eliminate foreign substances from attacking the body and initiating numerous diseases.[45]

The immunomodulatory activites of *C. asiatica* extract were being studied by various researchers *in-vitro* and *in-vivo* assays. The immunomodulatory activities of crude extracts and isolated fractions and bioactive compounds derived from *C. asiatica* have been depicted in Table 4. The preliminary study by Mali and Hatapakki identified that various concentrations of ethanolic leaves extract of *C. asiatica* 25, 50 and 100 mg/mL significantly increased migration activities of neutrophil cells from the upper compartment to lower surface of filter in a dose dependent manner. The similar effect of extracts on phagocytosis activities of neutrophils which was evaluated by slide method showed that the extracts also enhanced the phagocytic index of neutrophil cells when compared to the untreated group. Next, the intracellular killing properties of neutrophils that treated with these various extract concentrations indicated more higher than negative control samples containing phosphate buffer saline solution when measured using qualitative nitroblue tetrazolium test.[46]

The other study by Pandiyan and team which used rabbit erythrocytes as an *in vitro* model presented that whole plants aqueous extract of *C. asiatica* at concentration of 15.63 to 1000 μg/mL significantly increased alternative pathway (AP) activity in a dose response relationship when compared to levamisole as a positive control. The AP_{50} of this extract presented the value of 617.02 ± 16.40 μg/mL. In theory, alternative pathway of complement system that consists of soluble plasma proteins has distinct benefits of innate immunity against invading microbes. The immune response that involved in this pathway is rapid but does not contained immunological memory. Therefore, agent capable of stimulating alternative pathway selectively could be a better choice to apply as an adjuvant in the antibiotic therapy to treat infectious diseases.[3]

Futhermore, there were also numerous *in-vivo* studies determined the immunomodulatory effects of this medicinal plant. The study by Jayathirtha and Mishra seeks to discover immunized Swiss albino mice with sheep red blood cell suspension as a model showed that the methanolic extract of whole part of *C. asiatica* at high dose (500 mg/kg body weight (BW)) presented the significant increasing number in the total white blood count in treated-mice when compared to negative control

Table 4. Immunomodulatory activities of crude extracts, bioactive fractions and compounds derived from *Centella asiatica*

No.	Tested substances	Model used	Tested dose	Results	References
1.	Ethanol extract of leaves	Human neutrophil cells	25, 50 and 100 mg/mL	The concentration of extract 25 to 100 mg/mL increase chemotactic, phagocytic and intracellular killing potency of human neutrophil	46
2.	Methanol extract of whole plant	Immunized Swiss albino mice with sheep red blood cell suspension	100 to 500 mg/kg BW	All concentration of extracts increased the phagocytic index in dose-dependent manner and only 500 mg/kg BW concentration of extract enhanced total white blood cells count	47
3.	Aqueous extract of whole plants	Human peripheral blood mononuclear cells and BALB/C mice	10, 100, 200 µg/mL (*in-vitro*),10, 100, 300 mg/kg BW (*in-vivo*)	Increased lymphocytes proliferation in a dose- dependent manner and IgM and IgG antibodies production in100 mg/kg BW-treated mice	48
4.	Aqueous extract of whole plants	Rabbit erythrocyte suspension	15.625 to 1000 µg/mL (*in-vitro)*	The extract showed concentration dependent increase in alternate pathway activity of erythrocytes with AP 50 is 617.02 ± 16.4 µg/mL	3
5.	Ethanol extract of leaves	Balb/C mice (6-8 weeks old and 25-30 g weight) infected by *Listeria monocytogenes*	50, 150 and 450 mg/kg BW	The extract at 50 and 150 mg/kg BW dose showedsignificantly higher IFN-γ secretion compared to untreated group at day-2 and 4 after infection	49
6.	Methanol extract of leaves	Mice (2 weeks old) infected by *Salmonella typhi*	125, 250 and 500 mg/kg BW	The extract at 500 mg/kg BW dose showed increase pH phagocytosis activity of macrophage cells.	50
7	Ethanol extract of aerial parts	Immunized Swiss albino mice (20-25 g) with sheep red blood cells	100 mg/kg BW	Extract induced haemagglutination titre values after 14 days treatment	52
8.	Pulverization of aerialparts	Crossbred pigs (8 weeks old)	Conventional diet supplemented with 0.5%, 1%and 2%	Pigs fed with 1% and 2 % pulverized *C. asiatica* demostrated significantly reduced IL-10 levels afterthree-month feeding	51
9.	Titrated extract	*In-vivo*:phthalic anhydride-induced atopic dermatitis animal model	0.2% and 0.4% of extract (40 µg or 80 µg/cm2)	Inhibited mast cells and infiltration of inflammatory cells and expression of iNOS and COX-2, and NF-κB and secretion of TNF-α, IL-1, IL-6, and IgE.	58
		In- vitro: LPS stimulated murine macrophage cell line RAW264.7	1, 2, 5 µg/mL	Inhibit nitrite oxide production and expression of iNOS and COX-2, and NF-κB DNA binding activities	
10.	Triterpenoid saponin fraction	Wistar female rats (120-180g) induced wound infliction	25 mg/rat	Treatment decreased the IL-1β and NF-κB	54
11.	Asiatic acid	LPS-induced RAW 264.7 macrophage cells	40, 80 µM	Both concentration 40 and 80 µM reduced NO in dose-dependant manner	54
12.	Medacassic acid	CT26 cells-bearing mouse model	50 mg/kg BW	Induced CD4+ and CD8+ T- lymphocytes subpopulations and pro-inflammatory cytokines IFN-γ and IL-4	53
13.	Asiatic acid	LPS stimulated murine macrophage cell lineRAW264.7	30, 60, 120 µM	Reduced the production of NO, IL-6, IL-1β,TNF-αand protein and gene expression of iNOS in dose-dependent manner	57
14.	Asiatic acid	*In-vivo*: Male Sprague-Dawley rats (7 weeksold) induced periodontitis	100 mg/kg BW	IL-6 and IL- 8 expression levels significantly inhibited in LPS-stimulated gingival tissues	59
		In-vitro: LPS stimulated murine macrophagecell line RAW264.7 or human gingival fibroblast (HGF)	25, 50, 100 µM	IL-6, and IL-8 productions were significantly reduced by asiatic acid in both LPS-stimulated murine macrophage cell line RAW264.7 and HGF meanwhile PGE2 and NO secretion, p65 NF-κB phosphorylation were inhibited in LPS-stimulated HGF	
15.	Ethanolic, methanolic and aqueous extract ofwhole plant, asiaticosi-de and madecassoside	TPA-stimulated fibroblast cells	Extracts (30 g/mL), compounds (5 µg/mL)	Suppressed the TPA-induced production of PGE2 and inhibited both COX-1 and COX-2.	61
16.	Asiatic acid	LPS-induced inflammatory response in human corneal epithelial cells (HCECs).	20 µmol/L	IL-8 and IL-6, IL-1β,TNF-α and TGF-β mRNA expression levels were significantly reduced in inflammatory model. Content of reactive oxygen species also decreased.	60

group, using cyclophosphamide-induced myelosuppression assay. Meanwhile, the effects of extracts on a phagocytic activity of treated mice had been measured by carbon clearance test. The findings exhibited that all the various concentrations of extract significantly elicited the phagocytic index of treated mice in a dose-dependent manner. However, there was no significant increase in the level of antibody production in any group of mice treated with *C. asiatica* extract.[47]

In addition, research done by Punturee and groups mentioned that aqueous extract of *C. asiatica* increased the production of primary immunoglobulin M (IgM) and secondary immunoglobulin G (IgG) antibody responses of immunized mice. Meanwhile, for *in-vitro* study involved water extract of *C. asiatica* extract significantly increased lymphocyte proliferation induced by pokeweed mitogen (PWM) in a dose-response manner and only slightly increased lymphocyte proliferation induced by phytohemagglutinin (PHA) after 72 hours incubation period. Besides, water extract of *C. asiatica* at high concentration (500 μg/mL) increased lipopolysaccharide (LPS)-stimulated tumor necrosis factor-alpha (TNF-α) production as well as phytohemagglutinin (PHA)-stimulated interleukin-2 (IL-2) when measured using enzyme-linked immunosorbent assay (ELISA) analysis.[48]

The *in-vivo* investigation by Trapika and colleagues revealed that ethanol extract of *C. asiatica* leaves at dose of 50 and 150 mg/kg BW having immunostimulant potency by increasing interferon gamma (IFN-γ) secretions in the spleen of Balb/C mice infected by *Listeria monocytogenes* when compared to the untreated group that only received distill water. The measurement of IFN-γ level was using ELISA sandwich method. The action of this extracts might be due to the ability of the bioactive compounds in this plant extract able to modulate other pro-inflammatory cytokines such as tumor necrosis factor- alpha (TNF-α) and interleukin-12 (IL-12) that influenced the secretion of IFN-γ from natural killer cells when infection occurred.[49]

Moreover, *in-vivo* exploration by Besung and team who were using mice infected by *Salmonella typhi* as a model discovered that leaves methanolic extract of *C. asiatica* leaves (125, 250 and 500 mg/kg BW) enhanced phagocytic capacity of macrophages at dose response relationship. The highest phagocytic value of macrophages was observed after treatment with concentration of 500 mg/kg BW which presented 209.12 ± 26.17 cells per 50 macrophage when compared with untreated mice (103.12 ± 5.11). The morphological observations using Giemsa stained cells were done through 1000x total magnification of light microscope to compare between macrophage of treated and untreated groups. The outcomes showed that the macrophage treated with *C. asiatica* extract having irregular shapes, eccentric location of horseshoe-shaped nucleus and a lot of vesicle and vacuole of lysosomes. Meanwhile, the morphology of untreated macrophage cells appear small and having regular shapes.[50]

Application of pulverized *C. asiatica* combination (0.5%, 1% or 2%) with conventional diet in crossbred pigs exhibited comparable levels of serum interleukin-10 (IL-10) to control. In addition, 1% and 2% *C. asiatica* revealed significantly reduced serum IFN-γ levels when compared with untreated group after one month treatment. However, pigs fed with 1% and 2% *C. asiatica* showed significantly decreased serum IL-10 but not significantly in serum IFN-γ levels at three months prior treatment. The results indicate that *C. asiatica* has the potential to inhibit both pro-inflammatory cytokines and anti-inflammatory activities in crossbred pigs.[51] Siddiqui and his group were using immunized Swiss albino mice (20 - 25 g) with sheep red blood cells as a research model to evaluate the immunomodulatory activities of ethanol extract of aerial part *C. asiatica* at concentration of 100 mg/kg BW. Blood samples were collected from the retro-orbital plexus of each animal on day 10 and 14 after sensitization with sheep red blood cell (SRBC) on day 7. The findings showed that haemagglutination titre value in treatment group was significantly induced after 14 days treatment when compared to control group. The findings suggested that the extract able to enhance humoral antibody responses in immunized mice.[52]

C. asiatica contained abundant of bioactive fractions and compounds which have various pharmacological properties including immunomodulatory activities. The activity of madecassic acid on CD4+ and CD8+ T-lymphocytes subpopulations in tumor-bearing mice was studied by Zhang and friends using flow cytometry analysis. The findings revealed that medacassic acid exhibited significantly increased of CD4+ and CD8+ T-lymphocytes subpopulation and also enhanced the secretion of pro-inflammatory cytokines IFN-γ and IL-4 when compared to the untreated group. Thus, they suggested that the madecassic acid administration boosted the rat's immune defenses system against tumorigenesis via both T-helper 1 (Th1) and T-helper 2 (Th2) mediated immune response.[53] Mahmood and team reported that the nitrite oxide (NO) production of LPS-induced RAW 264.7 macrophage cells had shown an decreasing level after treatment with 40 and 80 μM asiatic acid. In contrast, at higher dose (160 μM), this compound exhibited the reverse phenomenon. NO released by macrophages was

detected using Griess assay.[54]

Inflammation is a normal physiology of our body's immune responses against infections. It includes the body's defend itself against foreign intruders such as viruses and bacteria, effort to heal itself after an injury and repair damaged tissue.[55] However, excessive inflammation gives augmentation to tissue damage, functional impairment, discomfort and pain.[56] *C. asiatica* also have an effect to modulate immune responses in various inflammatory model studies. Yun and colleagues revealed the effect of asiaticoside and asiatic acid (30, 60 and 120 μM) isolated from the leaves of *C. asiatica* on LPS-stimulated RAW 264.7 macrophage cells. The outcomes presented that asiatic acid dose-dependently reduced nitrite oxide and prostaglandin E2 (PGE_2) more potently when compared to asiaticoside. Next, the asiatic acid produced inhibitory activities in the production of pro-inflammatory cytokines of TNF-α, IL-6 and IL-1β in LPS-stimulated RAW 264.7 macrophage cells in a dose dependent manner. Later, the molecular studies reported that this compound inhibited protein and mRNA expression of inducible nitrite oxide synthase (iNOS) and COX-2, nuclear translocation of NF-κβ and phosphorylation of both inhibitor of nuclear factor of kappa light polypeptide gene enhancer in B-cells subunit alpha (IκB-α) and inhibitor of nuclear factor kappa-B kinase subunit beta and alpha (IKK-α/β). They suggested that the anti-inflammatory properties of asiatic acid might be the results from the inhibition of IL-6, IL-1β, iNOS, COX-2, and TNF-α expressions through the down-regulation of NF-κB activation due to inhibition of IKK and MAP kinase phosphorylation in RAW 264.7 cells.[57]

The activities of the titrated extract of *C. asiatica* (TECA) in a phthalic anhydride (PA)-induced atopic dermatitis (AD) animal model and LPS-stimulated RAW 264.7 macrophage cells were studied by Park and friends. This *in-vivo* studies exhibited a reduction in infiltration of inflammatory cells, mast cells and hyperkeratosis through histological analysis of ear and back skin of both groups of animal treated with 0.2% and 0.4% combination of PA and TECA. The expression of iNOS, COX-2, NF-κB activity and secretion of TNF-α, IL-1β, IL-6, and IgE in animal samples also significantly declined after treatment with 0.4% combination of PA and TECA. Meanwhile, *in-vitro* study showed that TECA at concentration of 1, 2, 5 μg/mL potently inhibited nitrite oxide released level and expression of iNOS, COX-2, and NF-κB DNA binding activities in LPS-stimulated RAW 264.7 macrophage cells.[58]

According to another research on the effects of triterpenoid saponins fraction (TSF) of this herb which applied on the wound area of Wistar female rat for 7 consecutive days demonstrated that TSF treatment decreased the expression of IL-1 β and NF-κB but increased the level of TNF-α when compared with the level of these similar parameter in the wound tissue.[54] There was a research conducted includes both *in-vitro* and *in-vivo* investigations using LPS-stimulated RAW 264.7 macrophage cells and isolated human gingival fibroblasts (HGFs) from explants of human normal gingival tissue and Male Sprague-Dawley rats (7 weeks old) induced periodontitis respectively. The methodology approaches by using ELISA and western blot to measure pro-inflammatory cytokines and protein expression levels respectively. The *in-vitro* findings validated that LPS-induced PGE_2, NO, IL-6, and IL-8 productions were significantly diminished by asiatic acid in a dose response manner after treating with compound at concentration of 25, 50 and 100 μM. Asiatic acid also reduced p65 NF-κB phosphorylation in LPS-stimulated HGFs. Meanwhile, the *in-vivo* study showed that LPS-induced NF-κB activation was inhibited by asiatic acid treatment.[59]

Based on the previous report, 20 μmol/L asiatic acid significantly inhibited the mRNA expression levels of IL-8, IL-6, IL-1β, and TNF-α in LPS-induced inflammatory responses in human corneal epithelial cells (HCECs) *in-vitro*.[60] In addition, another exploration on ethanolic, methanolic and aqueous extract of whole plant of *C. asiatica* at a concentration of 30 g/mL and both its' bioactive compounds (madecassoside and asiaticoside at 5 μg/mL) suppressed the 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced production of PGE_2 and inhibited both COX-1 and COX-2 in TPA-stimulated fibroblast cells.[61]

Despite of all the reports, there is still lack of data to offer verifications on immunomodulatory activity of this plant. Actually, adequate studies have not been performed on normal state model in both *in-vitro* and *in-vivo* studies regarding all parameters comprising pro-inflammatory cytokines, pro-inflammatory enzymes, proteins and genes expression. The assessment of the effects of pure compounds and fractions on the activity and gene expression of enzymes and cytokines involved in a normal model might be beneficial to investigate the ability of this herb as a potential to boost body's immune system as an initial prevention against invading pathogens that cause various ailments.

Conclusion

The consequences of the outcomes from all these

investigations suggest that *Centella asiatica* has a potential to be applied as a future anti-microbial and immunomodulator agent for developing new natural based pharmaceuticals to treat or prevent human pathogenic microorganisms that responsible to initiate diseases.

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