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Article

Spectroscopy Analysis of Antimicrobial Compound in *Centella Asiatica* Extract

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Abstract— **The usage of herbal medicinal is still the widespread among society today in their daily lives. This is mostly due to the tradition of our ancestors who really relied on traditional medicine to cure diseases and improve their intelligence. Next, herbal plants are said to have more and better nutrients and nourishment than modern medicine. This is because the compound have been mixed with the use of other chemicals to produce medicines. *Centella asiatica (pegaga)* is an herbal plant that is commonly heard about its benefits both internally and externally. However, scientific research is needed to prove its benefits. Thus, the society do not clarified much on the actual nutrients thoroughly. Besides that, *Centella asiatica* is generally known to come from the *Apiaceae* family and this plant is an important plant with a wide range of traditional, medicinal and therapeutic values. This plant has it own speciality or strong point in which it is choosen to be mentioned in the Quran. To prove its efficacy, a spectroscopy analysis of *Centella asiatica* extract was performed using ATR-FTIR. The use of *Centella asiatica* samples has also been diversified into leaf part and stem part. This is to compare the nutrient content in the leaves. The results from this research is that *Centella asiatica* leaves do have antimicrobial compound. Therefore, the usage of *Centella asiatica* leaves in society needs to be expanded so that its nutrients can be utilized to the maximum for the benefit of the people and improvement to the national economy.**

Keywords— ***Centella asiatica*; Antimicrobial properties; ATR-FTIR analysis; Functional group.**

I. INTRODUCTION

One of the most generally utilized therapeutic plants in Malaysia is *Centella asiatica*. A huge plant with a wide scope of customary, restorative, and helpful qualities, *Centella asiatica* (Linn.) has a place with the plant family Apiaceae (umbeliferae) (Zahara et. al., 2014). Around the world, this medicinal plant is available and is utilized as a wellspring of food, drink, and medication. *Centella asiatica* has been eminent for the treatment of wounds for a long time. It is additionally referred to in Malaysia as *pegaga*, in Europe and America as Indian pennywort and *gotu kola*, in India as *mandookaparni*, in Indonesia as *pegagan* or *kaki kuda*, and in China as *luei gong gen* or *tung chaim*. Notwithstanding being utilized as conventional and elective medication, *Centella asiatica* is generally utilized as a vegetable and beverages like tea in this area. It flourishes plentifully in moist zones and is a little, yearly herbaceous plant of the Mackinlaya sub-family, recently remembered for *Hydrocotyle*, that happens in Malaysia's muggy zones (James & Dubery, 2009).

Fig 1. *Centella asiatica* leaves

Centella asiatica has been ordinarily utilized in people' s medication for a long time to treat a wide assortment of infections. One such herb, widely used as a green leafy vegetable, is *Centella asiatica* (Gotu kola) in traditional societies around the globe because of its well-known health benefits. It has been highly regarded since prehistoric times for its use as a medicinal herb (Sabaragamuwa et. al., 2018). *Centella asiatica* has various pharmacological activities, such as wound healing, antimicrobial, anti-inflammatory, antioxidative, anti-diabetic, anti-depressive, anticancer, memory enhancing, neuroprotective properties. Vitamin C, vitamin B1, vitamin B2, niacin, carotene, and vitamin A are also rich in *Centella asiatica*. Chloride, sulphate, phosphorus, iron, calcium, magnesium, sodium, and potassium are found in the total ash (Seevaratnam et. al., 2012).

Centella asiatica likewise one of the therapeutic plants that show antimicrobial activities as it has potential in wound mending activities. Numerous therapeutic plants have antimicrobial properties, which means they can kill organisms like microbes, growths, parasites, and infections. As a remedy for the treatment of different diseases, including asthma, gastrointestinal indications, skin issues, respiratory and urinary issues, and hepatic and cardiovascular illness, therapeutic plants are generally utilized internationally (Tian et al., 2014). The structure of naturally dynamic restorative plant mix shifts generally, contingent upon the types of plants, the sort of soil, and their cooperation with microorganisms (Zhao et al., 2011; Morsy, 2014).

There are various active ingredients in *Centella asiatica* (L.) Urban, including triterpenoid saponins, triterpenoid genes, essential oils, flavonoids, phytosterols, and other active ingredients. Triterpenoid saponins are the most essential active component of many other active ingredients (Irham et. al., 2019). *Centella asiatica* is a precious plant that has historically been used for the treatment of a wide variety of diseases and has demonstrated immense medicinal and therapeutic benefits. In various commercial products, such as skin care, antiaging, slimming teas, and others, phytochemicals such as asciatic acid, madasiatic acid, betulinic acid, and thankunic acid are isolated from this plant are used (Zahara et. al, 2014). It is stated that *Centella asiatica* can be used for the treatment of leprosy, madness, asthma, ulcers, eczema, tuberculosis of the skin, wounds, stomach aches, arthritis, varicose veins, and high blood pressure (Rattanakom & Yasurin, 2014). The north-east Indian tribal people live in a rich area of biodiversity have a long herbal medicine history. *Centella asiatica* is used to treat high blood pressure as a liver tonic, blood purifier, anti-dysgenic, antiseptic, increased memory capacity, and high blood pressure medication (Nongkhlaw & Joshi, 2014). Natural products have various chemical properties that are capable of affecting the assessment of antifungal activity, such as the release of active ingredients, solubility, stability, absorption, and dissolution (Negri et. al, 2014). It has been scientifically proven that *Centella asiatica* herb can be useful in the treatment of skin diseases, especially in wound healing (Bylka et. al, 2014)

Centella asiatica leaves have several essential medicinal values that have not been scientifically established in the Malay community. Aside from its detoxifying activity, one of the common uses of *Centella asiatica* is its potential for wound healing activities. This research focuses on identifying antimicrobial compound in *Centella asiatica* using spectroscopy analysis. *Centella asiatica* will be collected and soaked for several days and is placed in a container in room temperature conditions.

A scientific procedure used to recognize natural, polymeric, and, in specific cases, inorganic materials is Fourier Transform Infrared Spectroscopy, otherwise called FTIR Analysis or FTIR Spectroscopy. Fourier Transform Infrared (ATR-FTIR) Attenuated Total Reflection Spectroscopy is a mark-free, non-dangerous strategy that can be applied to a wide assortment of natural applications, from imaging malignancy tissues and live cells to surveying the organization of protein substance and protein optional structure (Tiernan et. al., 2020). One of the most widely recognized testing methods in Fourier Transform Infrared (FTIR) spectroscopy is constricted absolute reflectance, or ATR, alongside transmission. ATR depends on complete internal reflection, where just at where IR light is reflected, infrared (IR) light and test connect. Attenuated Total Reflection (ATR) examining upset FTIR spectroscopy, with its straightforwardness of test-taking care of and close general pertinence.

To scan test samples and observe chemical properties, the FTIR analysis system uses infrared light. By producing an infrared absorption spectrum, it recognizes chemical bonds in a molecule. The spectra create a sample profile, a distinctive molecular fingerprint that can be used for several different

components for screening and scanning samples. The interpretation of FTIR spectra comes next once the initial testing and spectrum collection is complete. Usually, to classify the functional groups present, interpreting FTIR spectra begins at the high-frequency end. To classify the compound positively, the fingerprint regions are then analyzed. One of the main benefits of ATR-FTIR imaging is that, prior to spectral measurements, it needs minimal or no sample preparation. This is due to the fact that, for ATR measurements, the penetration depth of IR light in the sample is independent of sample thickness. The antimicrobial properties of *Centella asiatica* will be calculated by the identification of the antimicrobial compound using this spectroscopy analysis. The identification of the antimicrobial compound using this spectroscopy analysis will determine the antimicrobial properties in *Centella asiatica.*

II. MATERIAL & METHODOLOGY

A. Materials

Centella asiatica leaves, ethyl acetate, distilled water, filter paper (Whatman' s No 1 Filter Paper)

B. Preparation of materials

The *Centella asiatica* leaves was bought at market in Selangor, Malaysia. The leaves was dried at 80^0C for 2h in oven. Grind the leaves using pestle and mortar and put it in a beaker. 2 g of *Centella asiatica* leaves was weighed and put into two conical flask. For the solvent, ethyl acetate originated from United States was chosen.

C. Extraction of *Centella asiatica* leaves

40 ml of ethyl acetate was poured into the conical flask which contains the *Centella asiatica* leaves. 2 sample prepared for *Centella asiatica* leaf part. The conical flask was covered with aluminium foil and left for 24h in room temperature. After 24h, the sample was filtered into conical flask using Whatman' s No.1 filter paper. After that, ethyl acetate were added into the filtrate for extraction process. The extraction process was conducted using liquid-liquid extraction process. The remaining solvent was further removed using rotatory evaporator and the temperature is set at 76^0C.

Fig 2. *Centella asiatica* that has been grinded

Liquid-liquid extraction (LLE) employs water-immiscible solvents to extract analytes from aqueous solutions. This extraction technique can be used to purify compounds or to separate compound mixtures, such as when a reaction mixture (known as an extractive work-up) isolates a substance. Two immiscible stages, one of which is typically water and the other is typically an organic solvent, are the basic method for conducting a liquid-liquid extraction. The two phases are positioned in a device called a separatory funnel, and between the two phases, compounds in the system will be dispersed.

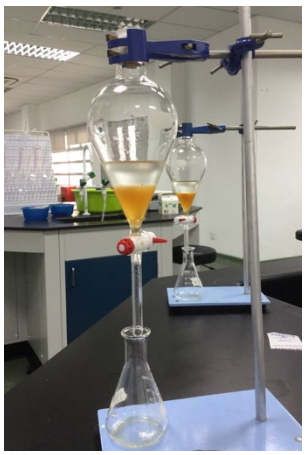

Fig 3. Liquid-liquid extraction process to obtain *Centella asiatica* extract

D. Characterization of antimicrobial compound using ATR-FTIR

Centella asiatica's examples were exposed to FTIR examination. The ethyl acetate extract was put straightforwardly on the precious stone window of the leaf part of the *Centella asiatica* leaf tests. In the north-south setup, all examples were situated and lined up with the examining shaft to take out ghastly varieties because of test area. Attenuated Total Reflection (ATR) is an inspecting procedure utilized in blend with infrared spectroscopy that permits tests to be legitimately analyzed without additional planning in the solid or liquid state. By estimating the progressions that happen in an inside reflected IR bar as the shaft comes into contact with an example, an ATR embellishment works. An IR bar at a specific point is guided onto an optically thick gem with a high refractive index.

The analysis was conducted using FT-IR spectrometer (Agilent Technologies, USA). MIR spectra were recorded from an accumulation of four scans in 4000 cm^{-1} to 600 cm^{-1} ranges. The accurate data was determined by the five relevant frequency ranges according to the IR windows. In each window, frequency range was adjusted to obtain a valid identification and determination in a test. Four scans spectrum of wave frequency from 3800 cm^{-1} to 800 cm^{-1} ranges were recorded. The average of the spectral was used for further study. Normalization and correction of background interference was performed on the spectrum. Spectrum transmission was obtained by using MicroLab software (Agilent Technologies, USA). Transmitted peak in ranges 4000 cm^{-1} to 600 cm^{-1} was generated in Microsoft Excel format (.xls) to facilitate the construction of graphs for further analysis.

III. RESULTS & DISCUSSION

Using spectroscopy analysis using ATR-FTIR, the functional group of antimicrobial compounds found in Centella asiatica leaves was determined. It will be identify through the spectrum and wavelength shown by ATR-FTIR. From that point, we can deduce the antimicrobial compound presents in *Centella asiatica* leaves through its functional group. Antimicrobial compounds in *Centella asiatica* leaves are cinnamaldehyde, eugenol, vanillin and safrole which indicates the antimicrobial properties in *Centella asiatica* (Bahrami et. al, 2020).

III. a. Fourier Transform Infrared (FTIR) Spectroscopy Analysis of *Centella asiatica* extract Sample 1

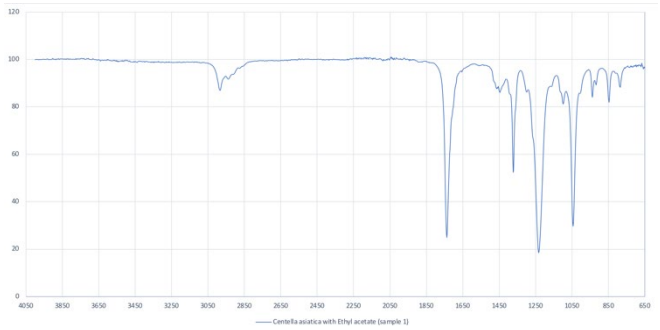

Fig 4. A spectrum graph of *Centella asiatica* extract Sample 1 obtained from ATR-FTIR

III. b. Fourier Transform Infrared (FTIR) Spectroscopy Analysis of *Centella asiatica* extract Sample 2

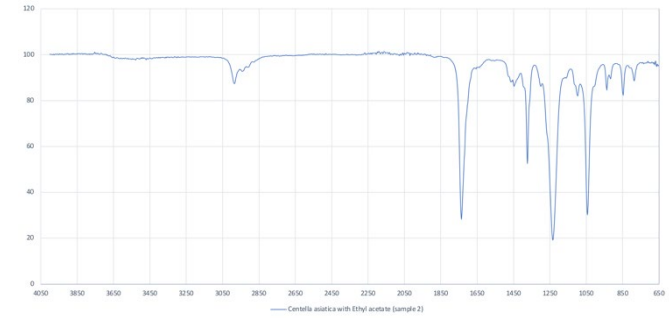

Fig 5. A spectrum graph of *Centella asiatica* extract Sample 2 obtained from ATR-FTIR

III. c. Overlay Graph of Fourier Transform Infrared (FTIR) Spectroscopy Analysis of *Centella asiatica* extract

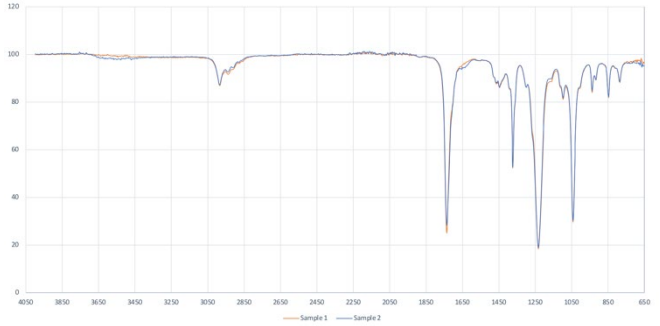

Fig 6. Overlay graph of *Centella asiatica* extract obtained from ATR-FTIR

III. c. The determination of active compounds using the presence of functional group

Ethyl acetate extract of leaf part of *Centella asiatica* leaves		
Frequency (cm^{-1})	Transmittance (%)	Functional group
1447.50	85.68	C–C stretch (in–ring) aromatics
1741.05	25.67	C=O stretch carbonyls (general)
2988.09	87.20	C–H stretch alkanes
1233.23	18.28	C–O stretch alcohols, carboxylic acids, esters, ethers
1098.24	80.96	C–N stretch aliphatic amines
2940.95	91.66	O–H stretch carboxylic acids
937.54	84.02	O–H bend carboxylic acids
849.69	82.34	C–Cl stretch alkyl halides

Table 1.0 shows the functional groups and compounds identified from sample

Using ATR-FTIR, functional group of compound present in the *Centella asiatica*' s leaves was determined. From the spectrum graph Fig 6., the functional groups which are the C-H stretch alkanes, C=O stretch carbonyls, C–C stretch (in–ring) aromatics, C-N stretch carboxylic acids, C-N stretch aliphatic amines, O-H bend carboxylic acids and C-Cl stretch alkyl halides and C–O stretch alcohols, carboxylic acids, esters, ethers are present. From this study, functional group of vanillin, cinnamaldehyde, eugenol and safrole was identified. MIR spectra of *Centella asiatica*' s extract was characterized by absorption peaks of various intensities in the wave−1 number range 3800 cm^{-1} to 800 cm^{-1} . Referring to Table 1.0, spectral absorption at 2940.95 cm^{-1} was assigned to O–H stretch carboxylic acids, 14470 cm^{-1} assigned to C–C stretch (in–ring) aromatics. While spectral absorption at 1741.05 cm^{-1} assigned to C=O stretch carbonyls (general) and 2988.09 cm^{-1} assigned to C–H stretch alkanes. Other than that, 1233.23 cm^{-1} assigned to C–O stretch alcohols, carboxylic acids, esters, ethers while 1098.24 cm^{-1} assigned to C–N stretch aliphatic amine. Next, spectral absorption at 937.54 cm^{-1} assigned to O–H bend carboxylic acids and 849.69 cm^{-1} assigned to C–Cl stretch alkyl halides. It can be seen that these functional groups existed in the *Centella asiatica*' s leaves. These functional groups are found in vanillin, cinnamaldehyde, eugenol and safrole which indicates the antimicrobial compounds. Thus, antimicrobial compunds are found in the *Centella asiatica*' s leaves which shows that *Centella asiatica*' s leaves has antimicrobial properties.

Numerous studies have attempted to explain about the usage of *Centella asiatica* as traditional medicinal herbs and its benefits. Moreover, there is an intimate and matchless relationship that has been formed over decades between indigenous people and their living environments. Based on their wealth of traditional expertise, they have built creative systems of practice to investigate and benefit from the biological diversity of these ecosystems. Futhermore, *Centella*

asiatica has been used for thousands of years in India, China, Sri Lanka, Nepal, and Madagascar as a medicinal herb. It is one of the major herbs for the treatment of skin problems, the healing of wounds and the revitalization of nerves and brain cells, primarily known in India as "brain food," and many body ailments (Chandrika & Kumara, 2016). *Centella asiatica* also known as pennywort and it has been said that pennywort from Thailand contained higher active compounds than the local pennywort (Ahmad et. al, 2016). One of the most potent herbal supplements for treating central nervous system (CNS)-related disorders is *Centella asiatica*. It has a mood-balancing effect and also improves emphasis (Belwal et. al, 2019). The *Centella asiatica* is a classical ethnomedicinal species used by ancient cultures and even by tribal groups. *Centella asiatica* is historically used in rubber and tea plantations in India and other Far East countries in the form of a cover crop. *Centella asiatica* is an effective treatment for different illnesses and has been used all over the world for thousands of years (Kant et.al, 2019).

Numerous studies have been done on antimicrobial compound in *Centella asiatica*. Antimicrobial activity against tested pathogens and antibiotic resistant strains was demonstrated by *Centella asiatica* extracts as an antibacterial and antifungal (Restuati & Diningrat, 2017). Based on previous studies, FTIR was used to classify the functional groups of the material investigated, which is shown by the peak absorbance rate. These FTIR spectra are more descriptive of maceral surface chemistry composition, while the presence of mineral peaks through the raw coal analysis affects the types and contents of the organic peaks (Wang et. al, 2017). The identity of compounds present in the extract of *Centella asiatica* should be assured by conventional medicine. The damaging compounds can be identified by this. The presence of the active molecules indicates that the *Centella asiatica* extract is safe (Rani et. al, 2019). The leaf and stem extracts of *Centella asiatica* contains alkaloids, proteins, flavonoids, terpenoids, tannins, steroids, saponins, reducing sugars, carbohydrates, amino acids, steroids. Main compounds observed include terpenoids and flavonoids, which are known to contain varied medicinal properties. The antimicrobial assays revealed that the extracts of *Centella asiatica* were active against S. aureus, K. pneumonia, E. coli and C. albicans (Ondeko et. al., 2020).

The preliminary phytochemical analysis of *Centella asiatica* leaf extracts were revealed the presence bioactive components namely alkaloids, amino acids, cardiac glycosides, phytosterols, triterpenoids, reducing sugars, steroids, saponins, flavonoids, phenols, tannins, anthraquinones in different concentrations except carbohydrates (Senthilkumar, 2018). The medicinal properties of *Centella asiatica* leaf extracts may be due to the presence of above mentioned phytochemicals. *Centella asiatica'*s hexane, dichloromethane, ethyl acetate, diethyl ether and methanol separates exhibited antibacterial movement against *B. K. subtilis*, *P. vulgaris*, *aerogenes* and *S.* types of *aureus* (Senthilkumar, 2018). Study by (Restuati et. Diningrat, 2018) stated that the concentrate of *Centella asiatica* ethanol is more successful as an antifungal than *P. pubescens*, where *Centella asiatica* successfully represses the development of *Aspergillus* and *fusarium*, yet *P. pubescens* is more compelling in hindering the development of *penicillium*. As an antibacterium, *Premna pubescens* is more powerful than *Centella asiatica*. *Premna* 's adequacy as an antibacterial is significantly more prominent than *Centella asiatica* for either Gram-positive or Gram-negative microorganisms. The concentrates of *Centella asiatica* are compelling in crushing microscopic organisms that can live under outrageous conditions, particularly *B.cereus*, for example, high or low temperatures (Utami et al., 2011). *Centella asiatica* has been utilized as an antibacterial operator in customary Malaysian medication and has been recommended as an option for skin infections and sensory system issues.

Utilizing Fourier Transform Infrared (FTIR) spectroscopy study, the substance structure of transformer paper in mineral oil was perceived. The new cellulose paper's FTIR spectra show ingestion tops that mirror an O-H useful gathering found near 3329 cm^{-1} (Munajad et. al., 2017). The current examination states, unexpectedly, that *C.gloeosporioides*, a foliar endophytic parasite of *Centella asiatica*, is creating asiaticoside. Up until the seventh subculture age, the endophytic organism had the option to deliver asiaticoside, proposing a likelihood that further enhancement studies could help in the huge scope advancement of asiaticoside. The high development rate and short age time show that endophytic growths might be a promising wellspring of creation of asiaticoside (Gupta et. al, 2018). Besides that, *Centella asiatica* is a necessary herb with a variety of healthcare applications. It is commonly recognized that plants have functions that are neuroprotective and beneficial in developing the brain. In clinical care with influential practices such as anticancer, antibacterial, antifungal, anti-inflammatory, neuroprotective, antioxidant, wound healing, and antidepressant, plants have been shown to be of low toxicity and greater efficacy (Prakash et. al, 2017).

IV. CONCLUSION

The spectroscopy analysis determined the antimicrobial properties of *Centella asiatica* using Fourier Transform Infrared Spectroscopy (FTIR) by interpreting FTIR spectra beginning at the high frequency end by the presence of functional groups. The result for this research is the presence of functional groups in *Centella asiatica* leaves are vanillin, cinnamaldehyde, eugenol and safrole which indicates as antimicrobial compound in *Centella asiatica*. Therefore, the usage of *Centella asiatica* leaves in society needs to be expanded so that its nutrients can be utilized to the maximum for the benefit of the people and improvement to the national economy.

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