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RESEARCH ARTICLE

COMPARISON OF DIFFERENT EXTRACTION METHODS TO STUDY THE ANTIMICROBIAL ACTIVITY OF CENTELLA ASIATICA LEAF EXTRACTS.

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Abstract

There are enormous bioactive compounds found in plants which serves as a major contribution in medicinal applications especially in treating diseases. *Centella asiatica* is one of the herbs that getting a great deal of consideration for its restorative purposes. This study was carried out to determine the different phytochemicals present in *Centella asiatica* leaf as well as its antimicrobial properties. The leaves were extracted with three different extraction methods; sonication, soxhlet, and maceration with ethyl acetate as a solvent. Sonication extraction gave the highest yield. Major groups of phytochemicals such as terpenoids, saponins, alkaloids and flavonoids were detected through qualitative and quantitative analysis. Antimicrobial analysis was done against two Gram- positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) and two Gram- negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*). There has been an increasing effect on microbial growth inhibition with increasing concentration of the extracts. Extracts from sonication had the highest inhibition rate for Gram-negative bacteria whereas maceration extracts had the highest inhibition rate for Gram-positive bacteria.

Introduction:-

Centella asiatica L. has been utilized as a therapeutic herb for a vast number of years in India, China, Sri Lanka, Nepal, Malaysia and Madagascar. It is one of the central herbs for treating skin issues, to recuperate wounds, for renewing the nerves and mind cells and subsequently known as a "Mind sustenance" in India (Saranya et al., 2017).

This plant is said to have antimicrobial properties. Fundamental bioactive constituents in *C. asiatica* is the triterpenes which comprise of asiaticoside, asiatic corrosive, madecassoside and madecassic corrosive have restorative incentive in different field. The developing concern with respect to the expansion of bacterial protection from anti-infection agents and expanding enthusiasm towards utilization of common medication have prompted the inquiry of new antimicrobial operators for the most part from plant remove (Farhana and Nadzir, 2017). Therefore, this study is aimed to analyse the antimicrobial properties of *C. asiatica* leaf by extracting the compounds with different extraction methods.

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Materials and Methods:-

Sample Collection

Fresh *C. asiatica* leaf were bought from local nursery, Seremban. Fresh fully matured leaf were washed thoroughly under running tap water and shade dried under the oven at the temperature of 30°C for 5 days. Then, the dry samples were homogenized to fine powder form.The dry weight and the moisture content was calculated.

2.2 Extraction Methods

2.2.1 Sonication Method (Angela and Nick, 2014)

Six grams of grinded *C. asiatica* leaf samples were extracted with 150 ml of 99% ethyl acetate. The samples were sonicated for 5 hours at a frequency of 40 kHz with a temperature of 70°C and ultrasound power of 90% (216 W). The filtered extracts were dried under the oven at 50°C.

2.2.2 Soxhlet Method (Richard et al., 2009)

Ten grams of grinded *C. asiatica* leaf sample was placed in the extraction thimble and 200 ml of 99% ethyl acetate was placed in the flask; the extraction process by the soxhlet apparatus was run for 5 hours. Then, the extract was filtered and dried under the oven at the temperature of 50°C.

2.2.3 Maceration Method (Bhavani Prasad, 2016)

Ten grams of grinded *C. asiatica* leaf samples were extracted with 250 ml of 99% ethyl acetate by placing in the incubator shaker for 7 days with occasional shaking. The extracts were then filtered and dried in the oven at a temperature of 50°C.

2.3 Phytochemicals Analysis

2.3.1 Test for Alkaloids (Prabhavathi et al., 2016)

To 2 ml of *C. asiatica* leaf extract, 2 ml of marquis reagent was added followed by 4 ml of concentrated sulphuric acid and a few drops of 40% formaldehyde. The appearance of dark orange or purple colour indicates the presence of alkaloids.

2.3.2 Test for Flavonoids (Krisnaiah et al., 2009)

A few drops of 1% ammonia solution was added to the *C. asiatica* leaf extract in a test tube. The formation of yellow colour indicates a positive results.

2.3.3 Test for Saponins (Krisnaiah et al., 2009)

2 ml of *C. asiatica* leaf extract was placed and then mixed with distilled water. Then the mixture was shaken vigorously for about a minute. Stable white foam or layer formation indicates the presence of saponins.

2.3.4 Test for Terpenoids (Krisnaiah et al., 2009)

2 ml of chloroform was added 2 ml of *C. asiatica* leaf extract followed by 3 ml of concentrated sulphuric acid solution. An interface with a reddish brown colour indicates the presence of terpenoids.

2.4 Quantitative Analysis

2.4.1 Alkaloid Determination (Harborne, 1973)

Five grams of the grinded *C. asiatica* leaf samples were added with 200 ml of 10% acetic acid in ethanol and allowed to stand for 4 hours. Then, the filtered extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution allowed to settle, and the precipitate was collected and washed with diluted ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried under the oven at the temperature of 50°C and then it was weighed.

2.4.2 Flavonoid Determination (Boham and Kocipai- Abyazan 1994)

Ten grams of the *C. asiatica* leaf sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through filter paper. The filtrate was later transferred into a falcon tube and evaporated into dryness under an oven and weighed to a constant weight.

2.4.3 Saponin Determination (Obadoni and Ochuko 2001)

Ten grams of grinded *C. asiatica* leaf sample was added with 50 ml of 20% aqueous ethanol. The samples were heated for 4 hours at 55°C. The mixture was filtered and the residue was re-extracted with 100 ml of 20% ethanol. The combined extracts were reduced to 40 ml over the water bath at the temperature of 90°C. The concentrate was transferred into a 250 ml separating funnel and 10 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated where 30 ml of n-butanol was added to the recovered layer. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven at 50°C to a constant weight and the saponin content was calculated as percentage.

2.4.5 Terpenoid Determination (Ferguson, 1956)

Ten grams of grinded *C. asiatica* leaf samples were soaked in alcohol for 24 hours. Then filtered, the filtrate was extracted with petroleum ether; the ether extract was treated as total terpenoids.

2.5 Determination of Antimicrobial activity by Disk Diffusion Method

Three different concentration of the extracts (15 mg/ml, 20 mg/ml, and 25 mg/ml) were tested for antimicrobial activity using disk diffusion assay according to the method by Farhana and Nadzir, 2017. The test microorganisms used in this study are; (Gram- positive and Gram- negative were used; *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Escherichia coli*). Bacteria plates was incubated at 37°C for 24 hours. The zone of inhibition was measured in millimetre (mm). Ampicillin (10µg) was used for *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* whereas the Streptomycin (10µg) was used for *Bacillus cereus*.

Results and Discussions:-

The dry weight and moisture content of the samples was 16.06% and 83.93%.

3.1 Extraction Methods

Sonication extraction method has given the highest yield which is 90% followed by soxhlet 87% and maceration 76%. Sonication method has given the highest yield because it applies ultrasound energy to agitate particles in the leaf sample (Azwanida, 2015).

3.2 Phytochemical Analysis

Table 1:-Phytochemical Screening of *C. asaitica* leaf

Phytochemical	Results
Alkaloid	Positive
Flavonoid	Positive
Saponin	Positive
Terpenoid	Positive

Results obtained for qualitative and quantitative of the phytochemicals in the leaf of *C. asaitica* are shown in table 1 and table 2.

Table 2:-Results of Quantitative Analysis

Phytochemicals	In grams	In %
Terpenoid	0.73	0.34
Saponin	1.02	0.47
Alkaloid	0.07	0.03
Flavanoid	0.23	0.11

Total amount of terpenoid present is 0.34% followed by saponin 0.47%, alkaloid 0.03%, and flavonoid 0.11%.

3.3 Antimicrobial Analysis

As the concentration of the *C. asiatica* leaf extract increases, zone of inhibition also increased. At the concentration of 25 mg/ml; the highest zone of inhibition which is 10 mm was recorded for *Staphylococcus aureus* using the leaf extract obtained from maceration extraction method followed by *Bacillus cereus*, 8.5 mm. The highest zone of

inhibition was 6.5 mm for *Pseudomonas aeruginosa* and 8.5 mm for *E. coli* using the leaf extract obtained from sonication.

The best extraction method which could inhibit the growth of Gram-negative bacteria is sonication whereas for Gram-positive bacteria is maceration. The cell wall of the leaf could be broken with the help of ultrasound through sonication extraction method and that is why it has a good results in inhibiting the growth of Gram-negative bacteria. According to Taemchuay et al., (2008), asiaticoside and asiatic acid are prominent in *C. asiatica* leaf that considered as the active ingredients of the plant. These compounds demonstrates its efficacy towards tested microorganisms such as *Staphylococcus aureus* and *Escherichia coli*. Rishikesh et al., (2012), stated that the tested microorganisms were inhibited by *C. asiatica* leaf extracts and this happens as a results of existence of tannins and glycosides in the leaf extract. These phytochemicals are reported to exhibit antimicrobial property in earlier studies. According to this statement, glycosides and tannins were detected to be present in this study through phytochemical screening.

Conclusion:-

Centella asiatica leaf was successfully extracted using ethyl acetate as a chemical solvent. Sonication extraction method showed the highest yield followed by soxhlet and then maceration extraction method. In phytochemical analysis, ethyl acetate had the capability to extract different phytochemicals present in the leaf such as saponin, terpenoids, alkaloids and flavonoids due to its high polar.

Antimicrobial analysis of *C. asiatica* leaf extracts had shown a good bactericidal effect against selected Gram-negative and Gram- positive bacteria and this depends on the susceptibility of that particular microorganisms towards the leaf extract. The best extraction method which could inhibit the growth of Gram- negative bacteria is sonication whereas for Gram- positive bacteria is maceration using ethyl acetate.

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