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(RESEARCH ARTICLE)

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Screening and evaluation of potential bioactive compounds for antibacterial activity in Indian medicinal plants of *Bacopa monnieri, Eclipta alba, Aegle marmelos* and *Centella asiatica*

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Abstract

The study was focused to identify and screening the potential action (antimicrobial activity) of ethanolic extract of the selected Indian medicinal plant leaves of *Bacopa monnieri, Eclipta alba, Aegle marmelos, Centella asiatica* against the following bacteria pathogenic isolates (*Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Enterococcus feceium , Enterobacter clocae , Pseudomonas aeruginosa and, Proteus mirabilis*). Leaves of the above mentioned Indian medicinal plants were shade dried, powdered, and extracted using solvent ethanol. The potential ingredients present in these plants are associated to the bioactive components possessing antibacterial property. The analysis of antibiotic property was executed using disc diffusion method and to determine the minimum inhibitory concentration of the plant leaf extracts. The two methods used in this study was that in the (method-1 the, disc were dried with plant extract and in the method-2 the, disc were soaked in the plant extract). The plant leaf extracts with potential bioactive compounds were screened using wavelength scanning in the range of 200nm to 900nm by UV-VIS spectrophotometer and their characteristic peaks were detected. Among the two methods, method -1 was found to be the best antibiotic activity among the tested medicinal plants. The disc diffusion method revealed a high degree of activity against bacterial clinical isolates. The UV-VIS profile showed different peaks ranging from 270nm-740nm with different absorption. The spectral analysis of *Eclipta alba, Bacopa monnieri, Aegle marmelos* and *Centella asiatica* revealed the presence of bioactive components and were answerable for the antibiotic activity against selected pathogens. From the result *Eclipta alba* was observed to be the best antibiotic activity while compared to other three medicinal plants. The results confirmed that these plants extracts were potential as a source of drugs to combat infections caused by prone bacteria. Thus helps to further studies to identify functional bioactive compounds responsible for the in vivo antibacterial activity and possible mechanism of action of the extracts.

Keywords: Antibacterial activity; Bioactive compounds; Metabolites; Indian Medicinal plants

1 Introduction

The medicinal plants are believed to be important source of first-hand chemical substances with prospective therapeutic effects. The secondary metabolites from plants were found to be the main source of various phytochemicals that could be directly used as intermediates for the production of innovative drugs. The natural medicines are believed to be more acceptable to the human body, when compare to modern synthetic drugs. The most important factor needed is to develop the supreme benefit from the traditional pattern of medicine for providing adequate healthcare service to all people. The wide range of medicinal plant parts are used for the

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extract which act as raw drugs and retain diverse medicinal assets (1). In India, the rich heritage of traditional knowledge and home-made to numerous significant time-honoured systems of health care practices include Ayurveda, Siddha and Unani are tailed. It has been assessed that the proportion of medicinal plants available in India (7,500 of the 17,000 higher plant species are medicinal plants) is higher than other country of the world with respect to the existing flora (2).

The plants include *Aegle marmelos (L.) Correa and Centella asiatica (Linn.)*Urban, are the two important and popular medicinal plants in India which have the antimicrobial activity. The medicinal significance of plants lies in some chemical substances that produce a positive physiological action on the human body. The most important bioactive compounds of these plants include alkaloids, flavanoids, tannins and phenolic compounds (3). Because of the prime benefits of using these plant derived medicines are comparatively safer than the synthetic alternatives, contributing deep therapeutic benefits and further reasonable treatment. The scarcity of infective disease in wild plants in itself is an indication of the successful defence mechanism developed by them. The defence mechanisms of the plants may be outstanding to the production of huge variety of small biomolecules generally classified as Phytoalexins (low molecular weight (MW< 500), antimicrobial compounds) (4). The compound phytoalexins, which has the structural spaces contain terpenoids, glycosteroids, flavonoids and polyphenols and these biologically active compounds supposed to express antimicrobial properties. These medicinal plants are the rich source of antimicrobial mediators (3, 5, 6). The plants of *Bacopa monnieri* (commonly known as brahmi) and *Eclipta alba* (commonly known as Kayunni) are the examples of medicinal plant which have the potential antibacterial activity (9,13). According to the world health organization (WHO), the medicinal plants would be the best source to obtain a variety of drugs in developed countries about 80 of plants are used in traditional medicine. The medicinal plants characterize a rich source of antimicrobial agents, and they are used medicinally in different countries for numerous potent and powerful drugs (6).

The screening and selection of pathogens based on the literature survey and report from the data, it was reported that the different kinds of pathogens were found in raw milk. They were listed from the dairy and clinical sources which includes *Escherichia coli, Salmonella, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococci pyogens, Klebsiellas, Microbacterium lacticum.* Usage of raw milk is quite common in our day to day activity. While using raw milk or even after boiling some of the pathogens remain in it and leads to some common infection along with other immune weakness either directly or indirectly. Hence, the aim of the study was to either kill or inhibit the growth of these pathogens by using antibiotic drugs produced from our common medicinal plants. The preferred medicinal plants of *Bacopa monnieri* and *Eclipta alba* are having the medicinal property of saponin (an antibiotic and antifungal agent). The medicinal property of *Centella asiatica* and *Aegle marmelos* are having the alkaloids, terpenoids and antibacterial agents. The rationale of this research work was to find out the antimicrobial and antibacterial property of the selected Indian medicinal plants [Bacopa monnieri (Brahmi), Eclipta alba (Bhringaraja, Kayyunni), Aegle marmelos (L.) Correa (Vilva), Centella asiatica (Linn.) Urban (Neer Bhrami, mandookaparni)] against the clinical bacterial pathogens (*E. coli, S. aureus, K. pneumonia, E. feceium, E. clocae, P. aeruginosa and, P. mirabilis*).

2 Material and methods

2.1 Plants material collection

The plants having healthy in nature with disease free of mature leaves (vegetative phase) of *Bacopa monnieri* (Fig. 1a) and *Eclipta alba* (Fig. 1b), *Centella asiatica* (Fig. 1c) and (Fig. 1d) *Aegle marmelos* were collected from *Kanhagad, Kasaragod*, Kerala. Then the leaves were both air dried and also dried kept in hot air oven at 50 ^{0} C temperature. Then the dried plant materials were ground into fine powder using motor and pestle, and subsequently sieved for obtaining fine powder.

2.2 Tested organism (Bacterial pathogens)

In this study, six bacterial clinical pathogens were used to find out the antibacterial activity of *Bacopa monnieri, Eclipta alba, Centella Asiatic* and *Aegle marmelos*. The bacterial pathogens of KDC strains include Escherichia coli, *Klebsiella pneumonia, Staphylococcus aureus, Enterobacter cloacae, Enterococcus faecium* and *Pseudomonas aeruginosa*. These clinical pathogens are were collected from KDC (Kanhagad Diagnostic Center) microbiology lab in Kanhagad, Kasaragod, Kerala.

Figure 1 Samples of (a) *Bacopa monnieri* , (b) *Eclipta alba* , (c) *Centella asiatica* and (d) *Aegle marmelos*

2.3 Plant extract preparation

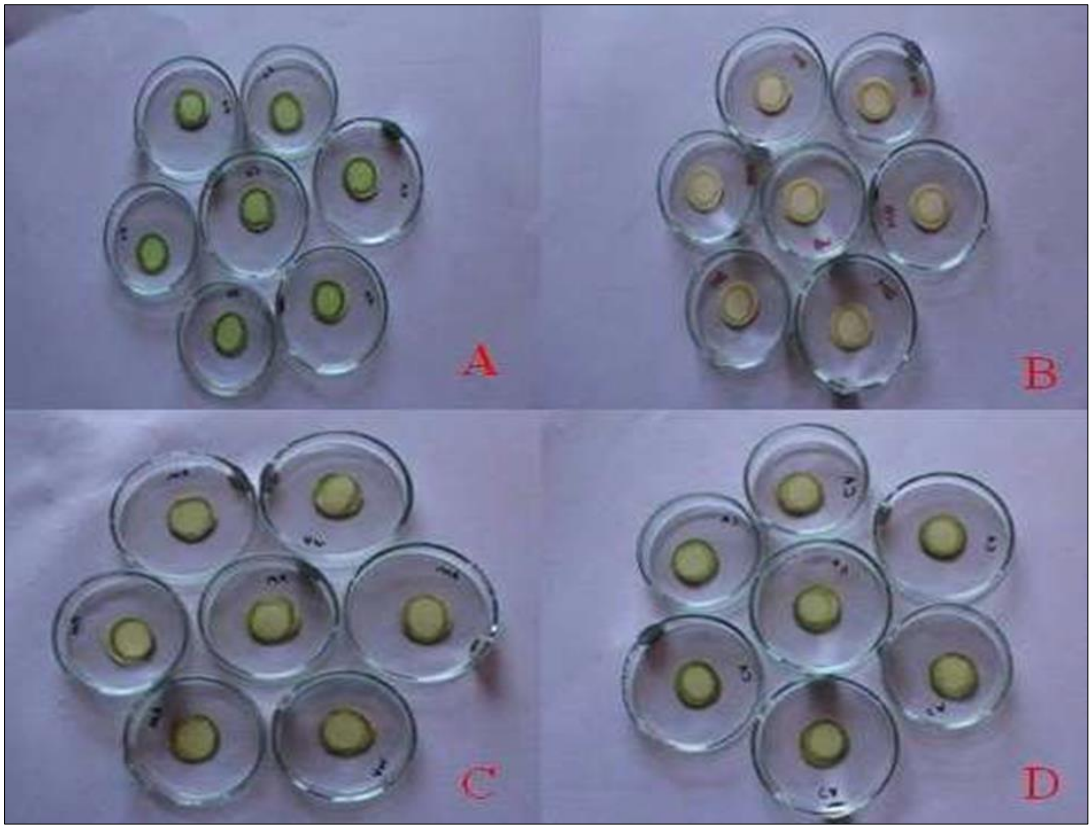

Figure 2 Discs dried with plant ethanolic extract (A) *Eclipta alba* and (B) *Bacopa monnieri* (C) *Aegle marmelos* and (D) *Centella asiatica*

The plant leaf material of 0.5 gm was weighed and sieved into fine powder and subjected to ethanolic extraction using 20 ml of 95% ethanol at room temperature and were centrifuged for 24 hrs at 5000 rpm. The extract obtained was stored in sample containers, Ethanolic extract of (BM) *Bacopa monnieri*(BM), *Eclipta alba* (EA), *Centella asiatica* (CA) and *Aegle marmelos* (AM) were collected and then concentrated plant extract were used for the antimicrobial assays (Figure 2).

2.4 Antibacterial activity test

The antibacterial potential was assessed by minimum inhibitory concentration (MIC) is the lowest concentration (1ml of phytoextract was used in this study) and able to inhibit any visible bacterial growth (bacterial counts of 40 x 105 CFU/µl in the present study). The MIC was determined and performed by the disc plate assay and the antimicrobial activity against bacteria was assessed by disc diffusion method (7).

2.5 Antibacterial activity (assay test) via disc diffusion method

The disc diffusion method was allowed for the concurrent testing of a large amount of antimicrobials in a relatively comfortable and flexible method. In this method, the inoculum were adjusted to certain concentration, and inoculated into the total surface of the nutrient agar plate with a sterile L-rod. Each of these discs having approximately 1.6cm in diameter was cut using the filter paper of Whatman No.1 type. The discs were put into a petri dish and then sterilized in the oven at 160 0C for 2 hrs. Then the discs were then impregnated with the extract by soaking in the leaf extract for upto 24 hours and each of these disc contained approximately 100 mg/ml of the ethanol extract. With the help of sterile forceps, each disc was then recovered from the extract, hold for a few seconds to evaporate some of the ethanol remain before being practically placed in aseptic condition onto the agar surface in a nutrient agar plate which had primarily being inoculated with a pure culture of test organism (100 µl). The control plates without the plant extract, using ethanol were performed as negative control, and the negative control (disc containing only ethanol) was consider in this study having zone formation against any organism for experimental comparison of ethanolic effect. The average colony forming units of 40 x 105 CFU/µl were taken for the investigation of antibiotic sensitivity tests (antibacterial assay) against the clinical bacterial pathogens by disc diffusion method. All the experiments in this study was carried out with triplicates.

2.6 Method 1: Disc dried with plant extract

The paper discs with 1.6 cm diameter are saturated wet with 1ml of plant ethanolic extract, which was added drop by drop over the dried disc. On each drop the disc was allowed to dry and then the dried disc was positioned on the surface of nutrient agar plate using sterile forceps. These plates were then incubated at room temperature, after 24hrs the zone of inhibition was observed and noted the dimension (Figure 2).

2.7 Method 2: Disc soaked with plant extract

The paper discs of 1.6 cm diameter were soaked or immersed fully in 1ml of plant ethanolic extract and allowed to dry completely. After drying, the dried disc containing plant extract was positioned on the surface of nutrient agar plate using sterile forceps. Then the plates were incubated at room temperature for 24hrs, the zone of inhibition was observed and noted the diameter value. The antimicrobial activity was evaluated by measuring the diameter of the inhibition zone formed, the experimental results were analysed through the mean values, mean of duplicates ± SD of the replication of experimental.

2.8 Bioactive compounds exploration by spectral analysis

The ethanolic plant extract was prepared and centrifuged at 5000rpm for 4hours and the samples were subjected to spectral analysis. The active fractional element of the plant leaf extract dissolved in ethanol were analysed for the presence of bioactive compounds by preliminary spectral analysis. The plant leaf extracts subjected to wavelength scanning in the range of 200nm -900nm, the spectral analysis with their characteristic peaks were detected for the presence of bioactive compounds using the UV-Vis Spectrophotometer (UV-2600, SHIMADZU).

3 Results and discussion

3.1 Antibacterial activity (assay test) via disc diffusion method

The serial dilution was performed for counting the bacterial colonies in each dilution. The dilution was done by pour plate technique using nutrient agar medium. The Table 1, represents the serial dilution of six clinical bacterial pathogens. The results were observed that the number of colonies forming unit was decreased when the dilution

rate increased. In the dilutions, for 10^{-1} and 10^{-2} dilutions the isolate of *Klebsiella pneumonia* was found to be countless since there was no countable colonies. As in the fifth dilution, the average colony forming units of 40X 10^5 CFU/µl were taken for the analysis of antibiotic sensitivity tests (antibacterial assay) against the bacterial pathogens by disc diffusion method.

Table 1 Serial dilution of clinical bacterial pathogens

Sample	Dilution factor	Average colony forming unit (CFU/µl)
Escherichia coli		
	10^1	356x10 CFU/µl
	10^2	180x10^2 CFU/µl
	10^3	128x10^3 CFU/µl
	10^4	80x10^4 CFU/µl
	10^5	40x10^5 CFU/µl
Staphylococcus aureus		
	10^1	352x10 CFU/µl
	10^2	280x10^2 CFU/µl
	10^3	108x10^3 CFU/µl
	10^4	76x10^4 CFU/µl
	10^5	40x10^5 CFU/µl
Klebsiella pneumoniae		
	10^1	Countless , Spreaded colony
	10^2	Countless, Spreaded colony
	10^3	372x10^3 CFU/µl
	10^4	156x10^4 CFU/µl
	10^5	40x10^5 CFU/µl
Enterococcus faecium		
	10^1	212x10 CFU/µl
	10^2	144x10^2 CFU/µl
	10^3	96x10^3 CFU/µl
	10^4	68x10^4 CFU/µl
	10^5	40x10^5 CFU/µl
Enterobacter cloacae		
	10^1	252x10 CFU/µl
	10^2	188x 102 CFU/µl
	10^3	120x10^3 CFU/µl
	10^4	60x10^4 CFU/µl
	10^5	40x10^5 CFU/µl
Pseudomanas aeruginosa		
	10^1	524x10 CFU/ µl
	10^2	392x10^2 CFU/ µl
	10^3	192x10^3 CFU/ µl
	10^4	68x10^4 CFU/ µl
	10^5	40x10^5 CFU/ µl

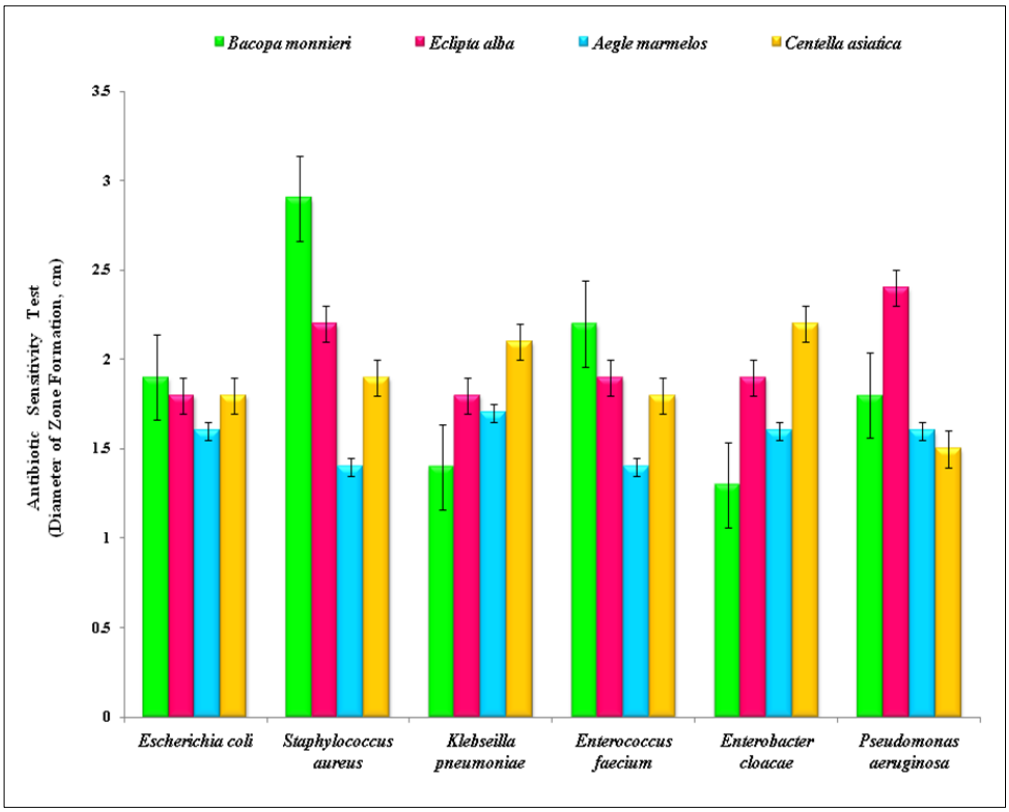

Figure 3 Antibiotic sensitivity test for Method-1 of Ethanolic leaf extract of medicinal plants against clinical bacterial pathogens (40 x 106 CFU/µl)

The figure 4 represents the antibiotic sensitivity test of *Bacopa monnieri, Eclipta alba Centella asiatia,* and *Aegle marmelos* and were studied against six clinical pathogens which includes *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Enterococcus faecium, Enterobacter cloacae and Pseudomonas aeruginosa*. As in the method 1, the discs were dried with plant extract and the disc diffusion method was performed. But in the method 2, the disc were soaked in 1ml of plant leaf extract, then dried and used for the analysis. The results revealed that the antibiotic sensitivity of each plant was increased according to increase in the dilution of broth culture of bacterial pathogens. The antibiotic sensitivity of each plant leaf extract was different against different microorganisms. The figure showed that highest antibiotic sensitivity (2.9cm) was at the dilution 10-5 ($40x10^5$ CFU/µl) by Staphylococcus aureus followed by Enterococcus faecium, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia and lowest value (1.3cm) by Enterobacter cloacae against the ethanolic extract of the plant leaves of Bacopa monnieri . In method 1 (figure 3) it exhibited that highest antibiotic sensitivity (2.4cm) at the dilution 10-5 ($40x10^5$ CFU/µl) by Pseudomonas aeruginosa followed by Staphylococcus aureus, Enterococcus faecium, Enterobacter cloacae and the lowest value (1.8cm) was observed in Escherichia coli and Klebsiella pneumonia against the ethanolic extract of the leaves of the plant Eclipta alba. The figure showed that highest antibiotic sensitivity (2.2cm) was at the dilution 10-5 ($40x10^5$ CFU/µl) by Enterobacter cloacae followed by Klebsiella pneumonia, Escherichia coli, Enterococcus faecium, and lowest value (1.5cm) noted in Pseudomonas aeruginosa against the ethanolic extract of *Centella asiatica*.

In method 2 (figure 4), showed the highest antibiotic sensitivity (1.7cm) at the dilution 10^{-5} ($40x10^5$ CFU/µl) by *Klebsiella pneumonia* followed by *Escherichia coli, Enterobacter cloacae, Pseudomonas aeruginosa.* The lowest value (1.4cm) was found in *Staphylococcus aureus* and *Enterococcus faecium* against the ethanolic extract of the plant leaves of *Aegle marmelos*. Similar trend was observed by Meenu (7) and Sharath Rajashekharappa (8), but the extract prepared was in water and methanol respectively. Among the plant extract, the *Bacopa monnieri* was observed to be high bioactivity against the pathogens that are tested in this study.

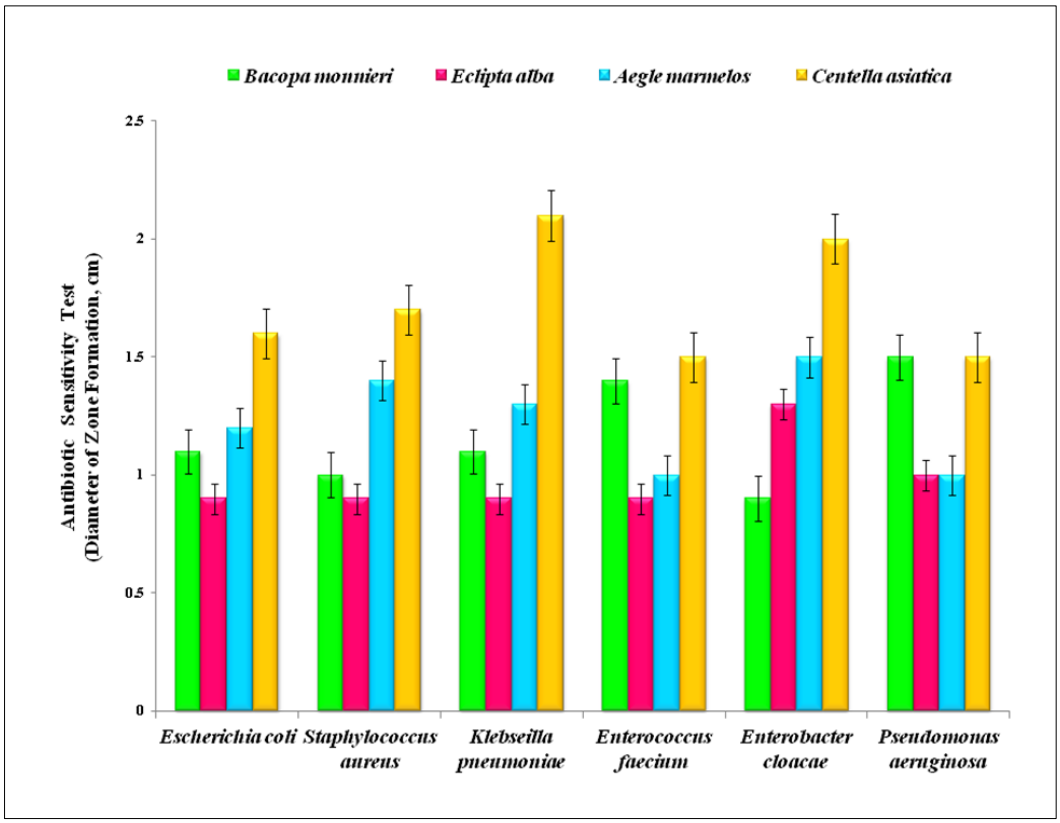

Figure 4 Antibiotic sensitivity test for Method-2 of Ethanolic leaf extract of medicinal plants against clinical bacterial pathogens (40 x 10^6 CFU/µl)

When comparing these two methods (based on the 10^{-5} dilution), the method 1 was found to be best when compared to method -2. From this study, it was observed that both these plants inhibit the growth of all the six clinical bacterial pathogens (Figure 5). These plants showed varied degrees of antibiotic activity against the tested clinical pathogens. *Bacopa monnieri, Eclipta alba Centella asiatia*, and *Aegle marmelos* showed more activity rate against the pathogens. Also, the study showed that *Staphylococcus aureus* was more sensitive to the plant *Bacopa monnieri*. This was followed by *Pseudomonas aeruginosa* which was more sensitive to *Eclipta alba* plant extract. Next to this, *Enterobacter cloacae* which was more sensitive to *Centella asiatica* followed by *Klebsiella pneumonia* and observed to be more sensitive to *Aegle marmelos*. The plant extract showed more resistant in the order of resistant that was observed to be first *Bacopa monnieri*, second *Eclipta alba,* third *Centella asiatia,* and the fourth *Aegle marmelos*.

The results observed were depend on the amended method, microorganisms, extraction procedure and their degree of solubility for each test compound (9). The medicinal plants have capability to synthesize aromatic substance with some endogenous metabolites. These substances served as a plant defence mechanism against predation by microbes, insects, herbivores, etc., (10). Some plants produced an assorted range of active biomolecules making them as a rich source of diverse kinds of drugs. The greatest pharmacological properties are because of the presence of the alkaloids and terpenoids existing in the respective medicinal plants.

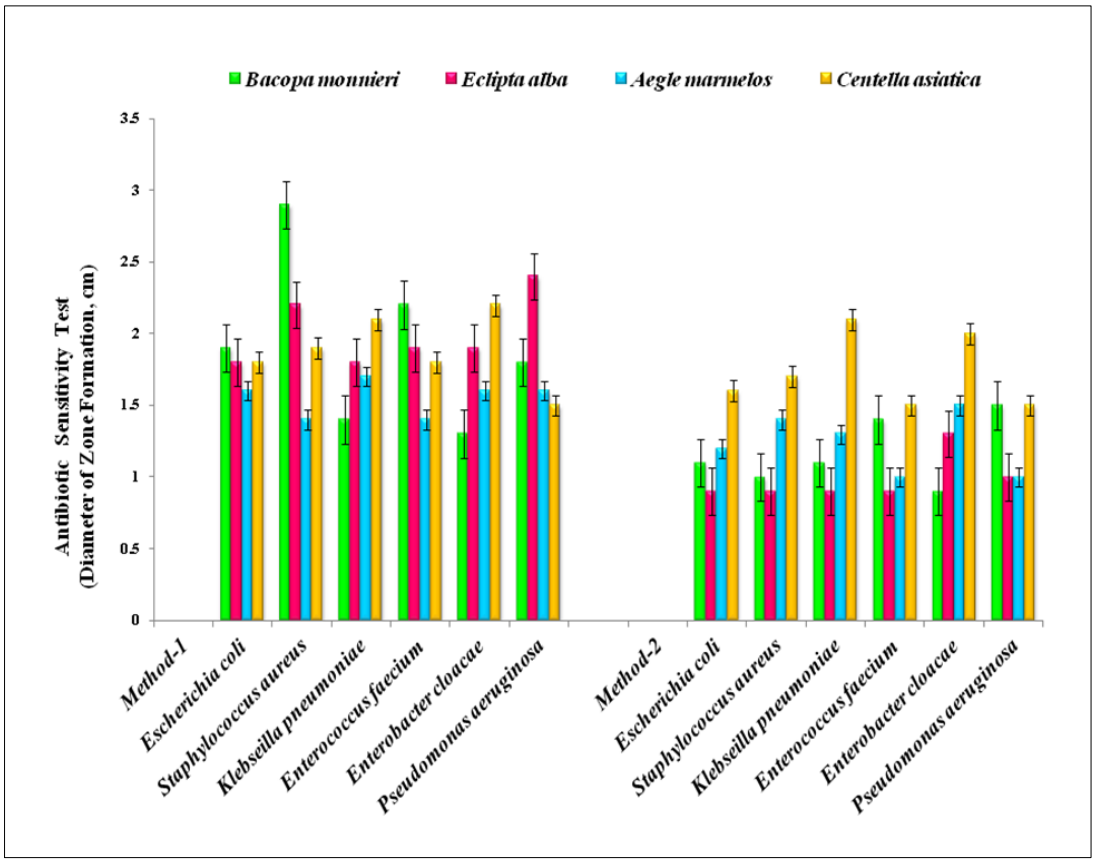

Figure 5 Antibiotic sensitivity test for Method-1 and Method-2 of Ethanolic leaf extract of medicinal plants against clinical bacterial pathogens (40 x 10^6 CFU/µl)

3.2 Bioactive compounds exploration by spectral analysis

The ethanol was a good solvent for phyto and polyphenol extractions. The extraction of bioactive compounds or herbal formulation, the best solvents used was the aqueous and hydro alcohol (70% or 50 % ethanol). Since these solvents can extract compounds from the herbal products, which was an excellent preservative and can also consume those substances/products without fright of toxicity. The alcohol affords a particularly an effective system of exploiting the bioavailability of these bioactive compounds extracted from the medicinal plant. The way of achievement that seems to be such that the alcohol acts to keep the bioactive components in the solution after ingestion and subsequently aiding their absorption into the blood.

The ethanolic leaf extract of these medicinal plants subjected to spectral analysis using UV-Visible Spectrophotometer for the detection of bioactive compounds in the respective plant leaves of *Bacopa monnieri*, *Eclipta alba*, *Centella asiatia*, and *Aegle marmelos*. The following bioactive compounds were present at different wavelength in these medicinal plants of *Bacopa monnieri*, *Eclipta alba*, *Centella asiatia*, and *Aegle marmelos* (Figure 6). The UV spectrum profile which showed different peaks ranging from 270nm to 740nm with different absorption points. The results of these UV spectral study were shown in Figure 5. The profile showed the characteristic absorption bands of bioactive compounds with different wavelength at 270, 330, 355, 410, 665 and 740 nm. Similar trend of UV-Vis profile was studied using benzene and chloroform extract by Johnson et al (11). The presence of the bioactive compounds were confirmed by the different peaks values which confirmed the presence of bioactive compounds in these plant leaf extracts and they were responsible for the antibiotic activity against the selected specific clinical bacterial pathogens. Fernandes et al (12), reported that the strong peak at 413 nm showed the main ingredient of the medicinal plant *Bauhinia monandra K*urz (Caesalpiniaceae). Similar statement was observed by Soundra pandian (13), stated that at 410 nm peak may be due to the flavonoid rutin with reference to the spectrum in *Centella asiatica* (Vallarai) (Figure 6), where rutin was shown as having a peak at 410 nm. Also he reported that the peak at 670 nm as well as the peaks down to 435 nm due to chlorophyll. Mona Kejariwal (14) observed that the UV-visible spectrum of standard quercetine was also recorded which gives peaks at 264nm, 250nm and 249 nm.

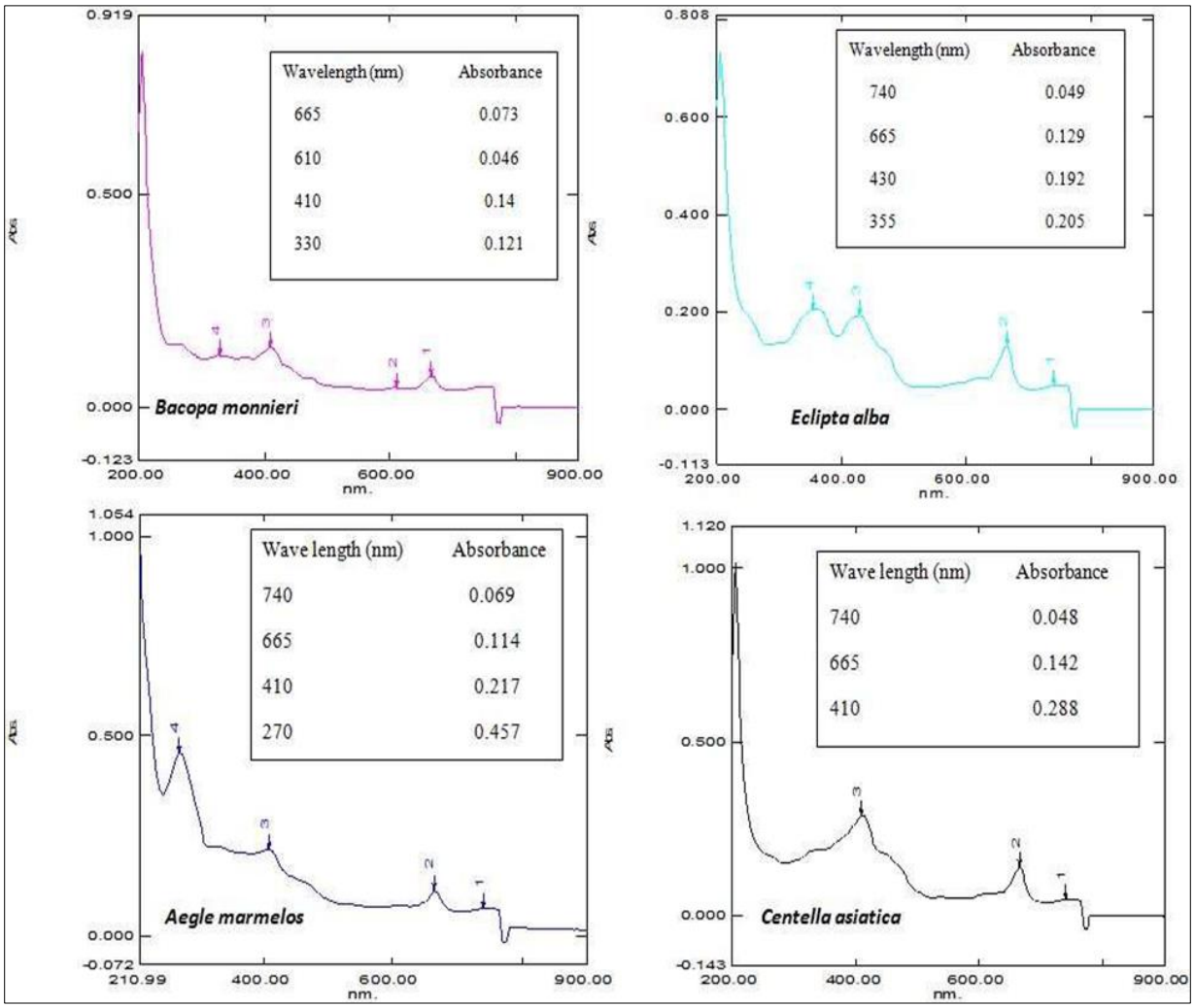

Figure 6 UV Spectrum of ethanolic extract of *Bacopa monnieri, Eclipta alba, Aegle marmelos* and *Centella asiatica* against the clinical pathogens

4 Conclusion

In conclusion, the *Bacopa monnieri* and *Eclipta alba* are active against the selected six clinical bacterial pathogens. From these result, the *Eclipta alba* was observed to be best activity while compared to *Bacopa monnieri*. Among the six clinical bacterial pathogens, *Eclipta alba* showed the greatest effect for four selective pathogens (*K. pneumonia, E. cloacae, P. aeruginosa, P. mirabilis*) and *B. monnieri* showed greatest result for three clinical bacterial pathogens (*E. coli, S aureus, E faecium*). Among the two methods adapted in this study, the antibiotic sensitivity of the selective plants was found to be better in method-1 (disc dried in plant extract) when compared to method-2 (disc soaked in plant extract). The spectral analysis of the ethanolic extract of *Eclipta alba, Bacopa monnieri, Aegle marmelos* and *Centella asiatica* showed the existence of bioactive molecules in their leaf extracts. Further they were answerable for the antibiotic activity test against the bacterial pathogens. This preliminary screening study helps in pharmacological activities and further studies needed to identify functional potential bioactive compounds responsible for the *in-vivo* antibacterial activity of the extracts.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there are no conflicts of interests regarding the publication of this manuscript.

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