Phytochemical Screening on Different Solvent of Selected Medicinal Plant Extracts Evaluated: Synergistic Antibacterial Activity of Ethanolic Plant Extracts Against Bacterial Pneumonia's Pathogens

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Abstract

In an attempt to find out the phytochemical screening for four medicinal plants like *Andrographis paniculate, Centella asiatica, Psidium guajava, and Solanum trilobatum* were studied. Five different solvents viz. petroleum ether, benzene, chloroform, and ethanol were used to obtain extracts from producing plant leaves. The extracts were subjected to qualitative phytochemical screening using standard procedure. The ethanolic plant extract was found to be more phytochemical than the other extract. The ethanolic extracts have been tested individually and in combination for their antimicrobial activity against *Streptococcus pneumoniae (mm)* and *Klebsiella pneumoniae* (mm) Strains. Results showed that the combination of plant extracts have higher antimicrobial activity than the individual plant extract. The four-plant extract ratio (1:1:1:1) of *Andrographis paniculate: Centella asiatica: Psidium guajava: Solanum trilobatum* was found to exhibit comparable activity (28 mm and 26 mm) than that of Amoxicillin (27 mm and 25 mm) and other combination (Two-plant and three-plant extracts) and individual plant extracts against *Streptococcus pneumoniae* and *Klebsiella pneumoniae.* Regarding the three-plant extract moderate antimicrobial activity against *Streptococcus pneumoniae* and *Klebsiella pneumoniae* were noticed.

Key words: Anti-pneumonia activity, phytochemical, synergistic activity, *Andrographis paniculate: Centella asiatica:Psidium guajava: Solanum trilobatum*

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Introduction

Plant materials continue to be a significant resource to prevent serious diseases. The traditional medicinal methods, particularly the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries. The medicinal value of these plants lies in some chemically active substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannin, flavonoid and phenolic compounds [1]. Within recent years, infections have increased to a great extent and antibiotics resistance effects become an ever-increasing therapeutic problem [2]. Natural products of the plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action [3,4]. *Andrographis paniculate*, native to Taiwan, Mainland China and India, is a medicinal herb with an extremely bitter taste used to treat liver disorders, bowel complaints of children, colic pain, common cold and upper respiratory tract infection [5,6]. The herb includes diterpenoids, flavonoids and polyphenols as the major bioactive components [7, 8]. Active compounds extracted with ethanol or methanol from the whole plant, leaf and stem [9] include over 20 diterpenoids and over ten flavonoids have been reported from *Andrographis paniculate* [10].

Centella asiatica, belonging to family Umbelliferae, also known as vallarai in tamil [11, 12]. It is a prostrate, slender, tender, faintly aromatic herb, which has numerous crawl stoloniferous stems, rooted at nodes with long internodes [13]. The plant contains the glycosides *viz.* Asiaticosides A & B, madecassosides and centellosides [14]. It contains the triterpene acids such as Asiatic and madecassic acid [15]. Flavanoids such as kaempferol and quercetin are also present in the plant [16]. The plant contains volatile and fatty oil. The fatty oil consists of glycerides of palmitic, stearic, lignoceric, oleic acids [17]. It is also rich in Vitamin C, Vitamin B_1, Vitamin B_2, niacin, carotene and Vitamin A. The total ash contains chloride, sulphate, phosphate, iron, calcium, magnesium, sodium, potassium etc [18, 19]. It has a long history of traditional use, a good proportion of which have been validated by scientific research [20]. The ethno-medicinal uses include the crushing of the leaves and the application of the extract on wounds, boils, skin and soft tissue infectious site [21]. The decoction or infusion of the leaves is used as febrifuge, antispasmodic and for rheumatism in India [22]. The leaves are used in USA as an antibiotic in the form of poultice or decoction for wounds, ulcers and tooth ache [23,24]. Bronchitis, asthma attacks, cough, pulmonary diseases could be also treated with guava teas [25]. *Solanum trilobatum* (Solanaceae) is a known shrub, familiar as 'Tuduvelai' and is distributed in various Asian countries, including

India, Sri Lanka, Indonesia, Singapore, and Malaysia [26]. In Ayurveda and Siddha medicinal systems, the roots and leaves are agreed to heal various respiratory tract problems, including acute and chronic bronchitis, asthma, sinusitis, tonsillitis, common cold, cough, and pulmonary infections [27]. The leaves are mainly used in treating dyspepsia, spermatorrhoea, tuberculosis, ear problems, and bacterial infections [28]. It has been extensively studied for various pharmacological activities including antibacterial, antifungal, anticancer, antioxidant, antidiabetic, hepatoprotective, antinociceptive, anti-ulcerogenic, and anti-inflammatory [29,30]. Studies showed that the leaves contain various metabolites, including sugar, fat, fiber, calcium, phosphorus, ferrous, and other minerals [31]. Phytochemical screening of the dried leaves contain alkaloids, triterpenoids, phenolics, tannins, flavonoids, anthoquinones, phytosterols, saponins, cardiac glycosides, soladunalinidine, tomatidine, solanine, sobatum, solasodine, diosgenin, and β-solamarine [32]. Nahrstedt and Butterweck [33] have been reported the traditional medicine, however, these compounds are mainly utilised as crude extracts in the form of herbal remedies. Herbal remedies are frequently prepared from a combination of several different plant species. The pharmacological effects of such mixtures could be as a result of the total sum of different classes of compounds with diverse mechanisms of action. In view of the above noticed facts and in continuation of our interest in the phytochemical screening on different solvent of selected medicinal plant extracts evaluated: Synergistic antibacterial activity of aqueous plant extracts against bacterial pneumonia's pathogens.

Materials and Methods

Plant Material

The leaves of *Andrographis paniculate, Centella asiatica, Psidium guajava,* and *Solanum trilobatum* were collected from Agastheeshwaram Taluk in Kanyakumari Dist.

Apparatus, Equipment, Chemicals and Consumables

The apparatus, equipment, chemicals and consumables used in our PG Chemistry and Zoology laboratory.

Extraction of leaves

Plant Sample Preparation

The leaves of the *Andrographis paniculate, Centella asiatica, Psidium guajava, and Solanum trilobatum* were washed thoroughly under tap water to remove dirt and shade dried

for ten days. The dried plant material was then powdered using a conventional blender and stored in a closed container [34].

Sequential Soxhlet Extraction of Plant Sample

Sequential Soxhlet extraction was used to extract out the phytochemical compounds contained in the leaves of *Andrographis paniculata* and *Centella asiatica.* The solvents with increasing polarity index (hexane, chloroform, ethyl acetate and methanol) was used for the extraction. A 25 g of powdered leaves was extracted with 250 ml of solvent by using a Soxhlet extractor for 4 hours [35].

Evaporation of Plant Extract

The crude solvent extracts obtained from the Soxhlet extraction were concentrated using a steam distillation and evaporator for the solvent. After that, the concentrated crude extracts were poured into a specimen vial [36]. The crude extracts were completely dried in a laboratory oven at 37°C. All the crude extracts were then weighed to determine the percentages yield of extraction and stored in refrigerator at 4°C.

Preliminary Phytochemicals Screening

Alkaloids Test

Each of the crude extract was dissolved in 1.0 ml of 1 % hydrochloric acid, HCl and treated with three drops of Dragendorff's reagent. Orange-red precipitation indicated the presence of the alkaloid compounds [37].

Phenols (Ferric chloride Test)

Each of the crude extract was added to 1.0 ml of 5 % ferric chloride, $FeCl_3$ solution. The blackish green color was indicated the presence of flavonoids [38].

Steroids Test

Each of the crude extract was dissolved in 1.0 ml of chloroform. A 2.0 ml of concentrated sulphuric acid, H_2SO_4 was slowly added to form a lower layer which is in yellow colour with green fluorescence. A reddish-brown colour on upper layer was interpreted as a steroid ring [39].

Glycosides Test

Each of the crude extract was dissolved in 1.0 ml of glacial acetic acid containing one drop of 1 % $FeCl_3$. This was followed by 2.0 ml concentrated H_2SO_4. A brown ring obtained indicates the presence of a deoxy sugar. A violet ring may appear below the ring. Meanwhile, a greenish colour ring might form in acetic acid layer and spread throughout in this layer [40].

Reducing Sugars Test

Each of the crude extract was mixed with 1.5 ml of distilled water to form an aqueous solution. Then, the extract was added to an equal volume of boiling Fehling A and B solution in separate test tubes. The presence of reducing sugars was interpreted as the formation of a brick red precipitate [41].

Tannins Test

Each of the crude extract was added to 2.0 ml of distilled water and boiled in a test tube and three drops of 0.1 % of $FeCl_3$ was added. The brownish green colouration indicates the presence of tannins [42].

Terpenoids (Salkowski Test)

Each of the crude extract was added to 0.4 ml of chloroform. A 0.6 ml of concentrated H_2SO_4 was added, two layers will be formed. The reddish-brown coloration in the interface has indicated the presence of terpenoids [43].

Saponins Test

Each of the crude extract was added with 1.0 ml of distilled water and boiled in test tube for 15 min. After cooling, the mixture was shaken and a persistent froth was indicated the presence of tannin [44].

Anthraquinones (Borntrager's Test)

Each of the crude extract was taken in a dry test tube and 1.0 ml of chloroform was added and shaken for 5 min. The extract was then shaken with equal volume of 10 % ammonia solution. A pink violet or red color in the ammoniacal layer (lower layer) has indicated the presence of anthraquinone [45].

Flavonoids (Alkaline Reagent Test)

Each of the crude extract was added to 5.0 ml of distilled water and boiled for 5 min. Three drops of 20 % NaOH solution were added. The colour change from colourless to yellow. Then, 5 drops of 1 % of HCl were added into the mixture. The presence of flavonoids was interpreted by observing the decolorization of the yellow colour [45].

Protein (Biuret Test)

One ml of 40% NaCl. and 2 drops of 1% CuSo4 were added to the leaf extracts. The appearance of a violet colour confirms the presence of proteins [46].

Agar Disc Diffusion Assay

Test Sample Preparation

A 100 mg of the solvent extracts was dissolved in 1.0 ml of solvent that used in extraction to make a concentration of 100 mg/ml. The methanol was used as negative controls.

Bacterial Suspension Preparation

Few colonies from the one-day old bacteria plate were inoculated into sterile 0.85 % saline water and the turbidity were adjusted to meet the 0.5 McFarland standard. To meet the 0.5 McFarland standard, the absorbance reading from UV visible spectrophotometer must be between 0.8 - 1.0 at a maximum wavelength of 625 nm [46].

Inoculate Bacterial Suspension onto Mueller Hinton Agar

The bacterial suspension was streaked on the MH agar by using a sterile cotton swab. The cotton swab was pressed firmly against the wall of the centrifuge tube to remove the excess fluid. The bacterial suspension was then streaked three times onto the surface of the agar in the different directions. This is to ensure that the bacterial suspension was distributed evenly on the agar [47].

Preparations and Application of Antimicrobial Discs

The prepared extract solution was sterile filtered using 0.45 μm membrane filter. After that, 10 μl of the extract solution was pipetted onto a 6 mm diameter sterile disc, which was made by Whatman filter paper No. 1 on a glass petri-dish. The disc containing the extract was allowed to dry in the laminar air flow for a few minutes. The disc was then placed gently on the MH agar plates that had been inoculated with the bacterial suspension using sterile forceps. A maximum of six discs can be placed on the MH agar plate, to avoid overlapping of the inhibition zones. In this study, there were two controls used which were solvent containing disc as the negative control and the commercially antibiotic containing disc (ampicillin) as the positive control for *Escherichia coli* and *Klebsiella Pneumonia*. A plate containing disc that's impregnated with extracts were incubated at 37 °C for 24 hours [48].

Recording Data and Interpreting the Results

The results were observed after 24 hours and the diameter of inhibition zone was measured by using a ruler in mm [48]. The study was done in triplicate and mean values were taken. Data was presented in the table format.

Results and Discussion

Sequential Extraction

From the Table 4.1 shows the percentage yield of extraction of the leaves of *Andrographis paniculate, Centella asiatica, Psidium guajava,* and *Solanum trilobatum* obtained using sequential extraction with a different polarity index of solvents. Results showed that the aqueous plant extract gave, the more percentage of phytochemical than other plant extract. The aqueous plant extract showed in the range in 18. 6 to 34.62%, followed by

ethyl alcohol (6.58 to 12.41%), chloroform (2.36 to 3.81 %) and Benzene (1.99 to 3.48 %). The percentage yield of extraction for petroleum ether was found to be lower (1.74 to 2.91 %). The *Solanum trilobatum* leaves more crude extract sample than the other plant extract.

Table 4. 1 Percentage of crude plant extract (50 g)

Plants	Plant extracts				
	Petroleum Ether (40-60°C)	**Benzene**	**Chloroform**	**Ethyl alcohol**	**Water**
Andrographis paniculata	1.74 %	1.99%	2.36%	6.58 %	18.60 %
Centella asiatica	2.55 %	3.43 %	3.45 %	11.22 %	30.32 %
Psidium guajava	3.07%	3.48%	3.94%	12.06%	34.27%
Solanum trilobatum	2.91%	3.44%	3.81%	12.41%	34.62%

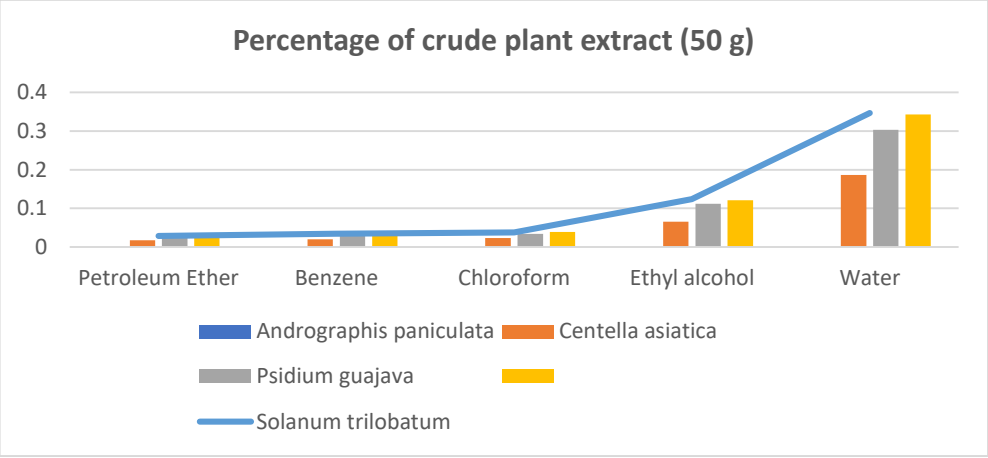

Fig. 4. 1 Percentage of crude plant Extract

FT-IR Analysis

The infrared spectroscopic analysis gives the information about the possible functional groups of active principles. The functional group analysis of the aqueous extract of *Andrographis paniculate*, *Centella asiatica*, *Psidium guajava*, and *Solanum trilobatum*, leaf were performed by using solid FT-IR spectroscopic analysis using the KBr disc method. Fig. 4.2 shows the FTIR spectrum of *Andrographis paniculate* leaves extract. The characteristic

absorption band were exhibited a 3323 cm^{-1} (for NH stretching), 3304 cm^{-1} (for OH stretching), 3242 cm^{-1} (for NH asymmetric stretching), 3194 cm^{-1} (for NH symmetric stretching), 1597 cm^{-1} (for C=O stretching), 1409 cm^{-1} (for CH stretching), 1201 cm^{-1} (for -C-O-C- stretching), 1041 cm^{-1} (for OH 1º alcohol) and 586 cm^{-1} (for C-Cl stretching) were exhibited by aqueous plant extract. FTIR spectrum of *Centella asiatica* leaves extract is showed in Fig. 4. 3. The characteristic absorption band were exhibited a 3323 cm^{-1} (for NH stretching), 3345 cm^{-1} (for OH stretching), 3242 cm^{-1} (for NH asymmetric stretching), 3194 cm^{-1} (for NH symmetric stretching), 1594 cm^{-1} (for C=O stretching), 1404 cm^{-1} (for CH bending), 1298 cm^{-1} (for CH stretching), 1044 (for OH 1º alcohol) and 565 cm^{-1} (for C-Cl stretching) were exhibited by aqueous plant extract. Fig. 4.4 shows the FTIR spectrum of *Psidium guajava* leaves extract. The characteristic absorption band were exhibited a 3340 cm^{-1} (for NH stretching), 3305 cm^{-1} (for OH stretching), 3252 cm^{-1} (for NH asymmetric stretching), 2954 cm^{-1} (for CH- CO stretching), 1584 cm^{-1} (for C=O stretching), 1392 cm^{-1} (for CH stretching), 1207 cm^{-1} (for -CH stretching), 1041 cm^{-1} (for OH 1º alcohol) and 557 cm^{-1} (for C-Cl stretching) were exhibited by aqueous plant extract. FTIR spectrum of *Solanum trilobatum* leaves extract showed in Fig. 4. 5. The characteristic absorption band were exhibited a 3345 cm^{-1} (for OH stretching), 3259 cm^{-1} (for NH asymmetric stretching), 3197 cm^{-1} (for NH symmetric stretching), 1594 cm^{-1} (for C=O stretching), 1404 cm^{-1} (for CH bending), 1265 cm^{-1} (for CN stretching), 1074 (for CH stretching) and 678 cm^{-1} (for C-Br stretching) were exhibited by aqueous plant extract.

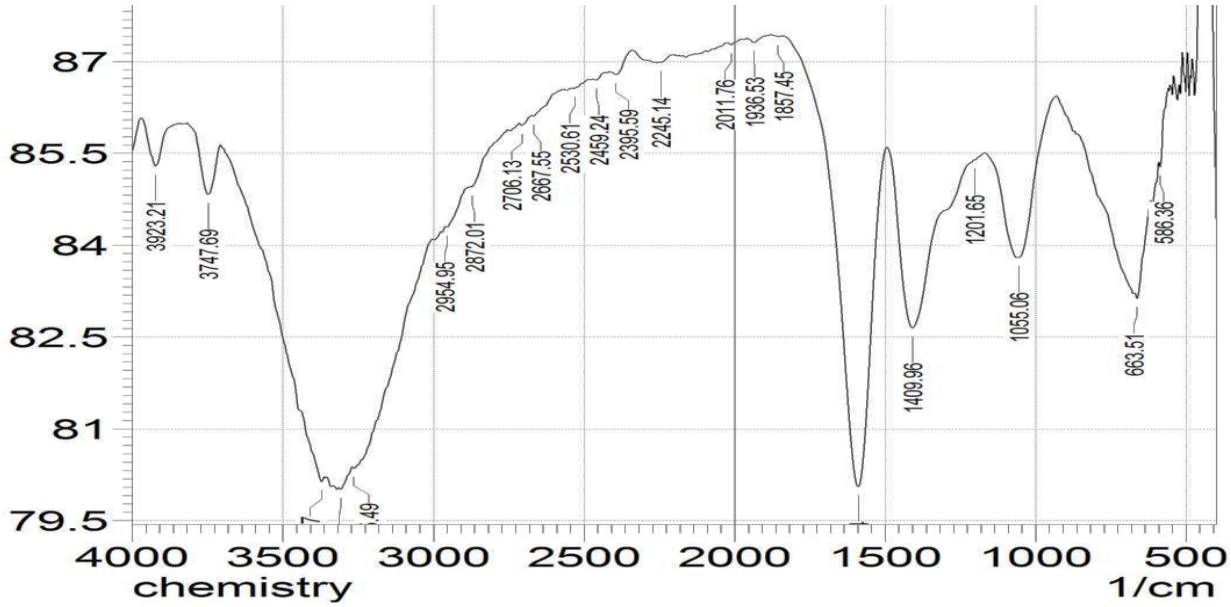

Fig. 4. 2 FTIR spectrum of *Andrographis paniculate* leaves aqueous extract

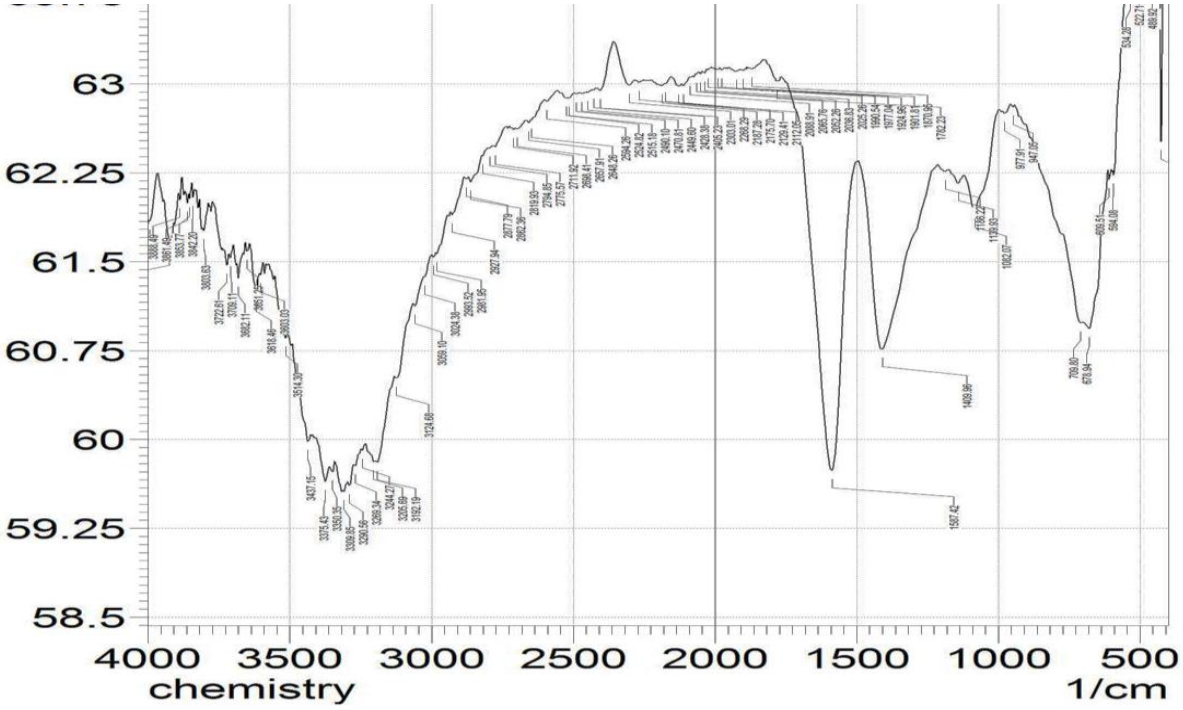

Fig. 4. 3 FTIR spectrum of *Centella asiatica* leaves aqueous extract

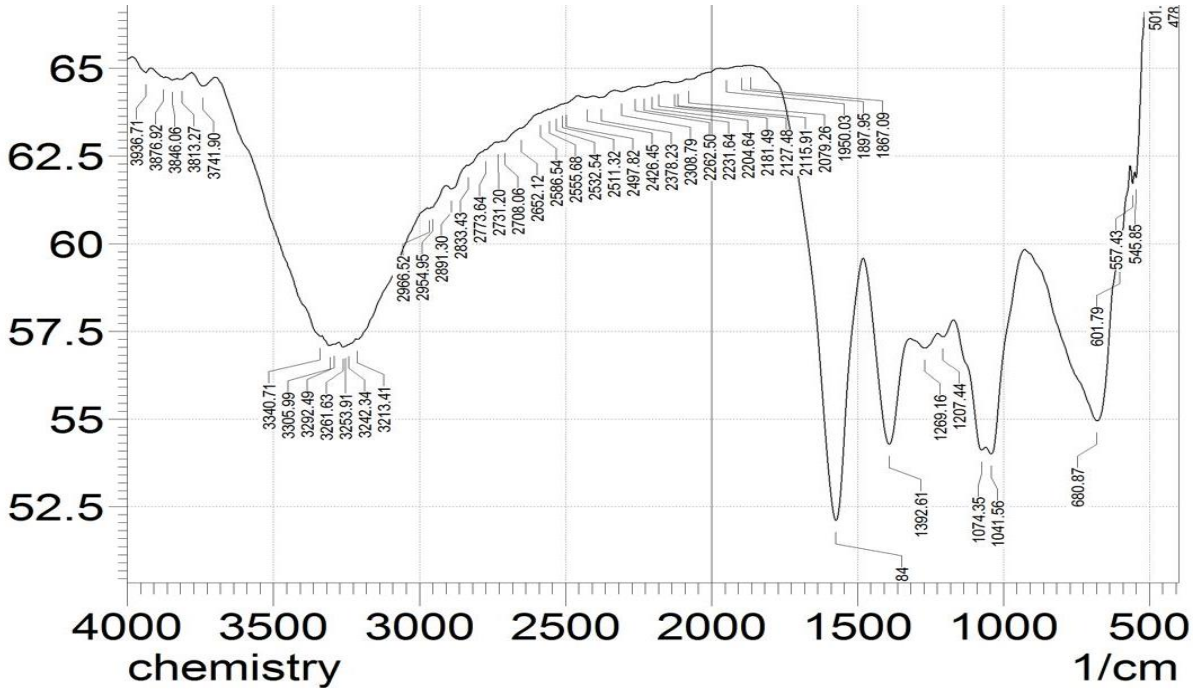

Fig. 4. 4 FTIR spectrum of *Psidium guajava* leaves aqueous extract

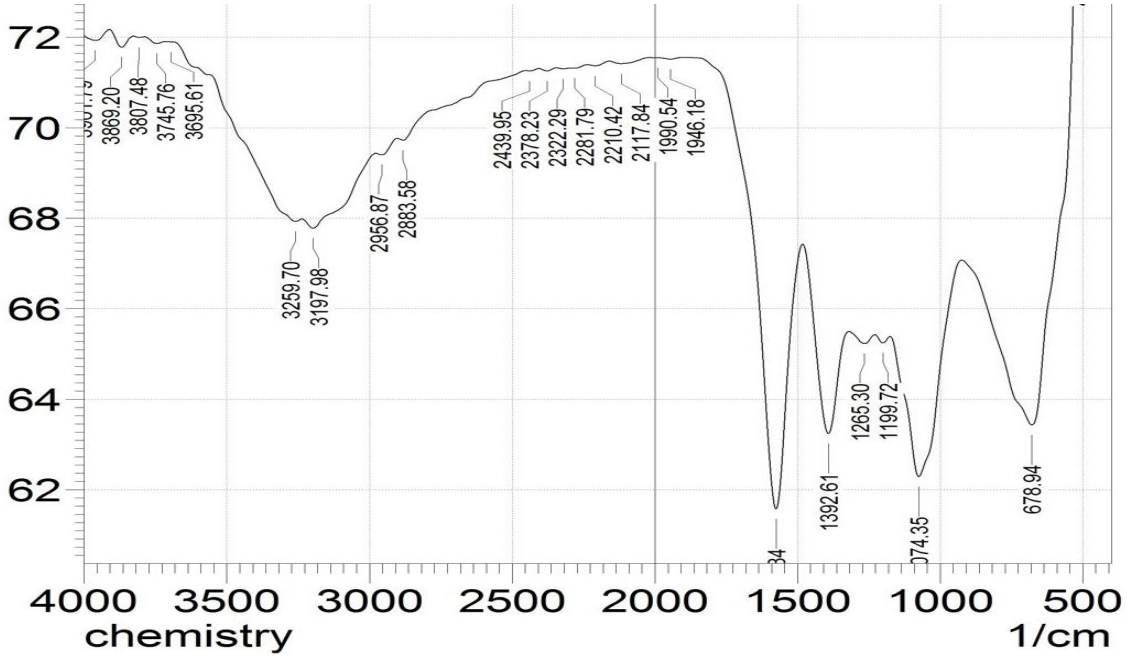

Fig. 4.5 FTIR spectrum of *Solanum trilobatum* leaves aqueous extract

4. 2 Phytochemical Analysis

Table 4.2 to 4.4 shows the qualitative analysis of the phytochemicals content in *Andrographis paniculate, Centella asiatica, Psidium guajava,* and *Solanum trilobatum* leaf extracts. According to Table 4.2 to 4.4, the aqueous extract of the *Andrographis paniculate, Centella asiatica, Psidium guajava,* and *Solanum trilobatum* contained the most of the phytochemicals compounds which are Phenols, Steroids Glycosides, Tannins, Terpenoids, Saponins, Alkaloids, proteins and flavonoids in maximum amount. The Chloroform and benzene extracts showed moderate amount of phytochemical such as Tannins, Terpenoids, Saponins, Alkaloids, proteins and flavonoids. The petroleum ether extract showed minimum number of phytochemicals such as Tannins, Terpenoids, flavonoids, Saponins, and proteins.

The *Andrographis paniculate* plant extract showed the phytochemical present as shown in Table 4.2. The phytochemicals Flavonoids, Saponins, and proteins are present in minimum amount in Petroleum ether extract. Similarly, Tannins, Terpenoids, flavonoids, and proteins are present in minimum amount of present in benzene and chloroform extract. The phytochemical of Phenols, Glycosides, Tannins, Terpenoids, Saponins, proteins and

flavonoids have a moderate amount of presence in ethanol extract and maximum amount in aqueous extract. Table 4.3 indicates the phytochemical present in the *Centella asiatica* plant extract. The tannins and flavonoids were present in the minimum amount in petroleum ether extract. Similarly, benzene and chloroform plant extract have minimum amount of tannins, flavonoids and alkaloids. The *Psidium guajava* plant extract showed the phytochemical present in Table 4.4. The phytochemicals of Tannins and Flavonoids are present minimum amount in Petroleum ether extract. Similarly, Tannins, Terpenoids, flavonoids, and proteins have a minimum amount of presence in benzene and chloroform extract. The phytochemical of Phenols, Glycosides, Tannins, Terpenoids, Saponins, proteins and flavonoids have a moderate amount of presence in ethanol extract and maximum amount present in aqueous extract. Table 4.5 indicates the phytochemical present in the *Solanum trilobatum* plant extract. The tannins flavonoids and protein were present in minimum amount in petroleum ether extract. Similarly, benzene and chloroform plant extract have minimum amount of tannins, terpenoids, flavonoids alkaloids and protein. The phytochemical of Phenols, reducing sugars, Tannins, Terpenoids, Saponins, proteins and flavonoids have a moderate amount of presence in ethanol extract and maximum amounce present in aqueous extract.

Table 4. 2 Phytochemical tests carried out the *Andrographis paniculate* plant extract in five different solvent

Phytochemical Testes	Petroleum Ether (40-60°C)	Benzene	Chloroform	Ethyl alcohol	Water
Phenols	-	-	-	+++	+
Steroids	-	-	-	++	+
Glycosides	+	-		+++	++
Reducing Sugars	-	-	-	-	-
Tannins	-	+	+	+++	+
Terpenoids	++	+	-	++	+
Saponins	+	-	-	+	++
Anthraquinones	-	-	-	-	-
Flavonoids	+	+	+	++	++
Alkaloids	-	-	+	++	+
Protein	+	+	+	++	++

*- Not present, + Minimum amount present, ++ moderate amount present, +++ maximum amount present

Table 4. 3 Phytochemical tests carried out the *Centella asiatica* plant extract in five different solvent

Phytochemical Testes	Petroleum Ether (40-60ºC)	Benzene	Chloroform	Ethyl alcohol	Water
Phenols	-	-		++	++
Steroids	-	-	-	-	--
Glycosides	-	-		+	+
Reducing Sugars	-	-	-	-	-
Tannins	+	+	+	+++	++
Terpenoids	-	+	+	+++	+
Saponins	-	-	-	+	++
Anthraquinones	-	-	-	-	-
Flavonoids	+	+	+	+	+
Alkaloids	-	+	+	+++	+
Protein	-	-	-	-	+

*- Not present, + Minimum amount present, ++ moderate amount present, +++ maximum amount present

Table 4. 4 Phytochemical tests carried out the *Psidium guajava* plant extract in five different solvent

Phytochemical Testes	Petroleum Ether (40-60ºC)	Benzene	Chloroform	Ethyl alcohol	Water
Phenols	-	-	-	++	+
Steroids	-	-	-	-	++
Glycosides	-	-		++	++
Reducing Sugars	-	-	-	-	-
Tannins	+	+	+	+++	+
Terpenoids	-	+	+	+++	+
Saponins	-	-	-	+++	+
Anthraquinones	-	-	-	-	-
Flavonoids	+	+	+	++	+
Alkaloids	-	-	-	-	-
Protein	-	+	+	++	++

*- Not present, + Minimum amount present, ++ moderate amount present, +++ maximum amount present

Table 4. 4 Phytochemical tests carried out the *Solanum trilobatum* plant extract in five different solvent

Phytochemical Testes	Petroleum Ether (40-60ºC)	Benzene	Chloroform	Ethyl alcohol	Water
Phenols	-	-	-	++	+++
Steroids	-	-	-	-	-
Glycosides	-	-	-	-	-
Reducing Sugars	-	-	-	++	++
Tannins	+	+	+	+++	++
Terpenoids	-	-	-	++	++
Saponins	-	-	-	+	++
Anthraquinones	-	-	-	-	-
Flavonoids	+	+	+	++	+
Alkaloids	-	+	+	+	+
Protein	+	+	+	+	++

*- Not present, + Minimum amount present, ++ moderate amount present, +++ maximum amount present

Phytochemical process was carried out among the four medicinal plant extracted with different solvents were processed to determine the phytochemical constitute [49]. The phytochemical process of the five solvent extracts was carried out to detect the presence of secondary metabolite such as flavonoid, tannins, terpenoid, steroid, alkaloid, and saponins, using the standard phytochemical method as reported by Sofowora [50]. This test to indicate the presence of various bioactive secondary product, which would be responsible for their five common plant attributes. Phytochemicals such as alkaloids, terpenoid, steroids, saponins were processed the standard methods, phytochemical analysis of plant was needed to discover and extended to novel therapeutic agents with improved efficiency. In this process deals with the secondary, based on phytochemical tests of *Andrographis paniculate, Centella asiatica, Psidium guajava,* and *Solanum trilobatum*, were contain some specific phytoconstituents.

Phytochemicals in greenery food had great deals of attraction. Mainly on their role in preventing diseases caused and the result of oxidative stress, and release reactive oxygen species have single oxygen of various radicals as a damaging side effect of aerobic metabolism. The detailed information of phytochemicals in various solvent were used to the process are shown in the above-mentioned tables. This paper mainly revealed that the phytochemical as secondary metabolite and they can be used to the pharmaceutical industry

for producing an efficient drug. This study indicating the result of the above medicinal plants gives a basis of application in traditional medicine, and also contain some bioactivity of phytochemical constituents was more valuable. Qualitative analysis of phytochemical was more interesting area and also an important application of biomedical in pharmaceutical industries. This phytochemical analysis was very useful in finding chemical compound in the plant material that led to their quantitative estimation and locating the pharmacy field [50].

Screening of four medicinal plants was an analysis to maximum classes of phyto-constituents are present. The medicinal plants have highest therapeutic efficiencies of pharmaceutical field. The five medicinal plants extract to indicate the more positive result of ethanolic plant extract than the other nonpolar solvents. Therefore, the ethanolic plant extracts were to determine the presence of Phyto-constituents. The medicinal plants have been used in the treatment of so many diseases and their medicinal roles of these plants have such a secondary product and identified the bioactive compounds.

Antimicrobial evaluation

The leaves of *Andrographis paniculate, Centella asiatica, Psidium guajava,* and *Solanum trilobatum* aqueous extracts were evaluated for their *in vitro* antibacterial activity against *Klebsiella pneumonia* and *Streptococcus pneumoniae* as examples of Gram-negative bacteria. Agar-diffusion method was used for the determination of the preliminary antibacterial. *Amoxicillin* was used as a reference drug. The results were recorded for each tested compound as the average diameter of inhibition zones (IZ) of bacterial growth around the discs in mm^3 and the inhibition zone diameter values are recorded in Table 3.1.

Table 4. 5 Inhibition zone (mm) of Plant Extract

Extract No	Plant Extract	*Streptococcus pneumoniae (mm)*	*Klebsiella pneumoniae (mm)*
1	*Andrographis paniculata*	15	18
2	*Centella asiatica*	13	15
3	*Psidium guajava*	10	12
4	*Solanum trilobatum*	16	19
5	*Andrographis paniculata: Centella asiatica (1:1)*	17	16
6	*Andrographis paniculata: Psidium guajava (1:1)*	15	17
7	*Andrographis paniculata: Solanum trilobatum (1:1)*	18	21

8	*Centella asiatica: Psidium guajava (1:1)*	14	15
9	*Centella asiatica: Solanum trilobatum (1:1)*	16	18
10	*Psidium guajava: Solanum trilobatum (1:1)*	15	16
11	*Andrographis paniculate: Centella asiatica: Psidium guajava (1:1:1)*	22	19
12	*Centella asiatica: Psidium guajava: Solanum trilobatum (1:1:1)*	19	18
13	*Psidium guajava: Solanum trilobatum: Andrographis paniculata (1:1:1)*	23	20
14	*Solanum trilobatum: Andrographis paniculate: Centella asiatica (1:1:1)*	24	22
15	*Andrographis paniculate: Centella asiatica: Psidium guajava: Solanum trilobatum (1:1:1:1)*	28	26
Standard	*Amoxicillin*	27	25

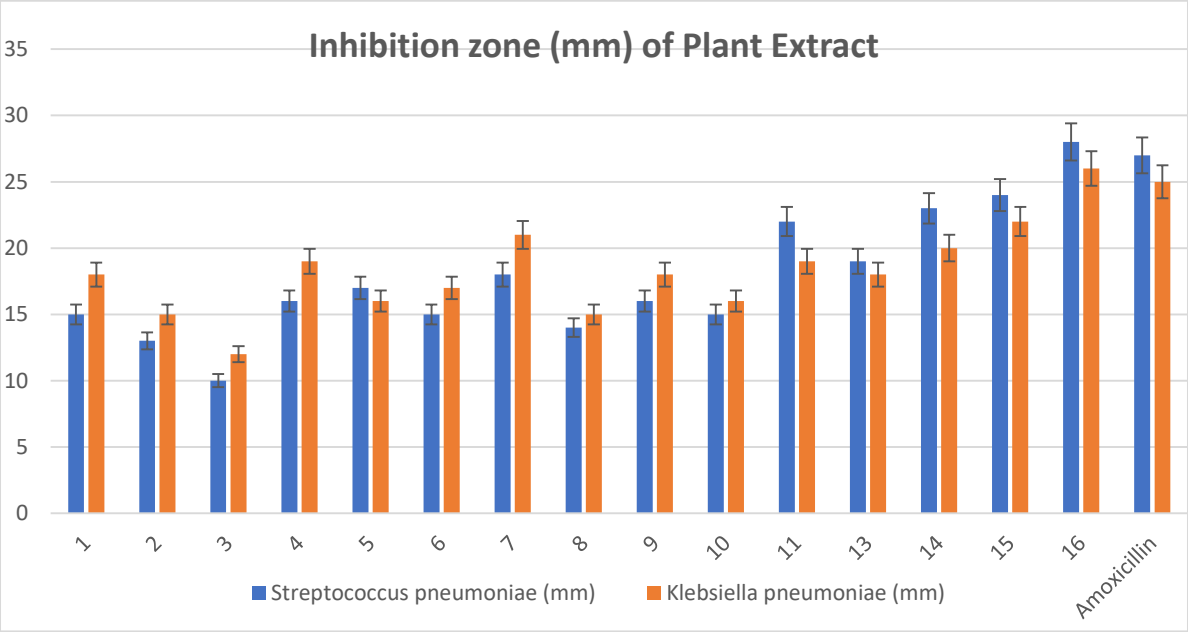

Fig. 4.5 Inhibition zone of Plant Extract

The results depicted in Table 4.5 revealed that most of the tested compounds displayed variable inhibitory effects on the growth of the tested bacterial strains like *Streptococcus pneumoniae* and *Klebsiella pneumoniae*. It would also be noticed that the two-plant extract combination of *Andrographis paniculata: Centella asiatica* (17 mm & 16 mm), *Andrographis paniculata: Psidium guajava* (15 mm & 17 mm), *Andrographis paniculata: Solanum trilobatum* (18 mm & 21 mm), *Centella asiatica: Psidium guajava* (14 mm & 15 mm),

Centella asiatica: Solanum trilobatum (16 mm & 18 mm), *Psidium guajava: Solanum trilobatum* (15 mm & 16 mm) extracts were exhibited higher antimicrobial potentials than individual extract**.** Similarly, three-plant extract of *Andrographis paniculate:Centella asiatica :Psidium guajava* (22 mm & 19 mm), *Centella asiatica:Psidium guajava: Solanum trilobatum* (19 mm & 18 mm), *Psidium guajava:Solanum trilobatum:Andrographis paniculate* (23 mm & 20 mm), *Solanum trilobatum: Andrographis paniculate: Centella asiatica* (24 mm & 22 mm) extracts were more antibacterial activity than the individual and two-plant (1:1) extract. The four-plant extract (1:1:1:1) ratio of *Andrographis paniculate: Centella asiatica:Psidium guajava: Solanum trilobatum* was found to exhibit combarable activity (28 mm and 26 mm) than that of Amoxycilin (27 mm and 25 mm) and other combination and individual plant extracts against *Streptococcus pneumoniae* and *Klebsiella pneumoniae.* Regarding the three-plant extract moderate antimicrobial activity against *Streptococcus pneumoniae* and *Klebsiella pneumoniae* were noticed.

Conclusion

This work concluded that the ethanol and aqueous extract of *Andrographis paniculate, Centella asiatica, Psidium guajava,* and *Solanum trilobatum* leaves gave the highest percentage yield of phytochemical. The study indicated that the leaf extract of *Andrographis paniculate, Centella asiatica, Psidium guajava, and Solanum trilobatum* contained more polar compounds than non-polar compounds. The phytochemical of Phenols, reducing sugars, Tannins, Terpenoids, Saponins, proteins and flavonoids have a moderate amount of presence in aqueous extract and maximum amount present in ethanol extract. The four-plant extract (1:1:1:1) ratio of *Andrographis paniculata: Centella asiatica* was found to exhibit higher activity (28 mm and 26 mm) than that of *Amoxicillin* (27 mm and 25 mm) and other combination and individual plant extracts against *Streptococcus pneumoniae* and *Klebsiella pneumoniae.* In this study clearly it is observed that the combination plant extract is higher potential than the individual extract.

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