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Characterization and Analysis of Medicinal Plant Extracts Against Nosocomial Infection

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ABSTRACT

The plant extracts are effective for the various aspects in the field of biology. There has been an increasing interest world-wide on therapeutic values of natural products. The extracts of *Trigonella* and *Phyllanthus* are observed to have anti-bacterial and anti-fungal activity. The clinical pathogens showed growth inhibition to the both extracts. The extracts showed inhibition of zone in *Staphylococci* and *E coli.* The combined effect was observed to have anti-fungal effect. The extracts were analysed for the anti-oxidant assays which showed fine amount of scavenging of the free radicals in total anti-oxidant capacity and DPPH assay. Thus, the concept will be useful for the treatment of some of the nosocomial infections.

Keywords: *Trigonella, Phyllanthus*, anti-microbial activity, anti-oxidant activity, nosocomial infection.

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INTRODUCTION

The nature had been a source of medicinal plants for thousands of years since the beginning of man, over the past twenty years; interest in medical plants has grown enormously from the use of herbal products as natural cosmetics and self-medication by the general public for their biological effects (Renu *et al* .,2016).

Phyllanthus has broad spectrum antibacterial activity on both gram positive and gram negative bacteria. A study carried out on different bacterial isolates; *Bacillus stearothermophilus, Staphylococcus aureus, Bacillus subtilis, Micrococcus leuteus, Salmonella typhi, Enterobacter aerogens, Proteus mirabilis,* and *Proteus vulgaris* revealed that *P. Amarus* showed the least MIC on all bacteria tested (kumar *et al.*, 2009). Similarly, the methanolic extract of *Phyllanthus amarus* was found to have potent inhibitory effect against drug-resistant pathogenic gram-negative bacteria; *Shigella* sp., *E. Coli, V. Cholerae, S. aureus, S. typhimurium, P. aeruginosa, B. subtilis, Klebsiella* and *Streptococcus sp.* In a dose-dependent manner (Laouer *et al* .,2009).

Phyllanthus niruri Linnaus., *Euphorbiaceae*, is sparsely spread throughout the tropical and subtropical countries of the world. This is an annual herb and widely spread in coastal areas of India **(**Mustofa *et al.,* 2007). *Phyllanthus* have broad spectrum of antibacterial activity on both gram positive and gram negative bacteria. A study carried out on different bacterial isolates; *Bacillus stearothermophilus, Staphylococcus aureus, Bacillus subtilis, Micrococcus leuteus, Salmonella typhi, Enterobacter aerogens, Proteus mirabilis,* and *Proteus vulgaris* revealed that *P. amarus* showed the least MIC on all bacteria tested. Similarly, the methanolic extract of *Phyllanthus amarus* was found to have potent inhibitory effect against drug-resistant pathogenic gram-negative bacteria; *Shigella* sp., *E. Coli, V. Cholerae, S. aureus, S. typhimurium, P. aeruginosa, B. subtilis, Klebsiella* and *Streptococcus sp.* in a dose-dependent manner (Babayi *et al.*, 2004).

MATERIALS AND METHODS

Collection of sample

Trigonella

Samples of *Trigonella* seeds were obtained from local market in Sivakasi. The samples were taken from retailer's store, in order to avoid the dust, dirt, loss of aroma and colour which may occur as a result of exposure of the spices to the direct sun light.

Phyllanthus

Fresh plant materials (keelanelli) were collected randomly from the western garden hills, Srivilliputtur, Virudhunagar and Tamil Nadu.

Preparations of seed extract (Priyanka *et al.,* 2011)

Trigonella

50gof dried and *Trigonella* seed powder was filled in jar. 300ml of methanol solvent was taken in flask. Temperature was maintained at the boiling point of respective methanol (620C – 720C) solvent.

Soxhelt extraction was continued till the solvent becomes colourless in tube. All the extracts were filtered through a whatman filter paper and then concentrated by using a hot air oven at low temperature 40 – 100°C. The extracts was collected and stored under dark in refrigerated conditions.

Preparation of leaves extraction in *Phyllanthus*

50g of *phyllanthus* samples were weighed and placed in thimbles for solvent methanol. Then, the thimbles were transferred into soxhlet extractors and cotton was inserted on the top of sample in thimbles to make sure that the sample will not spill out from the thimble during *phyllanthus* extraction. The extraction periods are 6 hours for solvent at 100°C. After the completion of extraction process, solvents were removed a rotary evaporator to obtain the crude extracts of *phyllanthus.*

Phytochemical analysis of the extracts (Kim *et al.,* 2006)

The two extracts of *Trigonella* and *Phyllanthus* was analyzed for the presence of certain phytochemicals. In every phytochemical analysis, methanol is mainly used as standard to identify various residues in the extract.

Alkaloids

To identify the presence of alkaloids in 2ml of extract is taken and to that 2ml of Wagner'S reagent is added. A brownish precipitation formation is observed. Thus it indicates the presence of alkaloids.

Cardiac glycosides

To test the presence of glycosides, 2ml of extract is dissolved with 2ml of chloroform then carefully add concentrated sulphuric acid to form a layer. Deep reddish brown colour at the interface of steroid ring indicates the presence of cardiac glycosides.

Flavonoids

To know the presence of flavonoids in the seeds, 2ml of extract is added to 2ml of 10% lead acetate. Yellowish green colour indicates the presence of flavonoids.

Saponins

For this, 2ml of extract is dissolved with 2ml of Benedict's reagent. Blue black precipitat indicates the presence of saponins.

Tannins

To know the presence of tannins, 2ml of extract is treated with 0.1% of Ferric chloride. Brownish green layer indicates the presence of tannins.

Terpenoids (Salkowski test)

To identify the presence of terpenoids, 2ml of extract is dissolved with 2ml of chloroform and concentrated sulphuric acid is carefully added to form a layer. A reddish brown colour is observed which indicates the presence of terpenoids.

Anthraquinones

To test the presence of anthraquinones in *Trigonella* seed extract, 1ml of extract is boiled with 10% HCl for few minutes in boiling water bath. Then it is filtered and allowed to cool. Equal volume of CHCl3 is added to the filterate and few drops of 10% Ammonia is added to the mixture and heat. A rose pink colour is found which indicates the presence of anthraquinones.

Reducing sugars

The extract was shaken with distilled water and filtered. The filterate is boiled with Fehling's solution A and B for few minutes an orange red precipitation indicates the presence of reducing sugars.

Glycosides

To identify this, extract is hydrolysed with HCl solution and neutralized with NaOH solution. Few drops of Fehling's solution A and B are added, Red Precepitation indicates the presence of glycosides.

Phlobatanins

The test the presence of Phlobatanins, the extract is dissolved in distilled water and filtered. The filtrate is boiled with 2% HCl solution. Red precepitation shows the presence of phlobatanins.

Characterization studies

Gas chromatography Mass spectrometry (Than *et al.*, 2006)

For GC-MS analysis 1µl of the sample is injected in split mode in the instrument. Use an Rtx5MS- 30m column. Following are the parameters standardized for GC-MS run: Injection temperature: 300°C, Interface temperature: 300°C, Ion source should be adjusted to 250°C. Carrier gas: Helium (flow rate of 1 ml min-1). Perform the analysis using the following temperature program: 1 min. of isothermal heating at 100^0C followed by heating at 300^0C for 20 mins. Mass spectra were recorded at 2 scan sec-1 with a scanning range of 40 to 850 m/z. Quantify each component based on peak areas and normalization based on the internal standard. Thus, GC-MS was performed for the both extracts to analyze the compounds present in the methanolic extracts.

Collection of Clinical sample

The patient sputum and urine was collected in the sterile container from government hospital at Sivakasi, Virudhunagar and TamilNadu.

Isolation of pathogen from clinical sample

The collected urine and sputum sample was streaked enriched media like MacConkey agar, Mannitol salt agar and Nutrient agar. The plates were incubated at 37oc for 24 hours. After the incubation period analyse the gram nature and morphology characterization. The biochemical characterization and identification of pathogenic bacteria species from urine and sputum samples were analysed.

Culture collection

The pure culture of *E.coli* and *Klebsiella* were collected from SMR laboratory at Sivakasi, Virudhunagar and Tamilnadu.

Biochemical characterization of the isolates

The biochemical characters like Indole test, Methyl red test, Voges- proskauer test, Citrate utilization test, Triple sugar iron test were performed for the isolates.

Determination of Anti-microbial activity (Maheswari *et al*., 2016)

The cultures were enriched in sterile nutrient broth for 6-8 hours at 37°C. Using sterile cotton swabs, the cultures were aseptically swabbed on the surface of sterile Muller Hinton agar plates, the different concentration of *Trigonella* extract and *phyllanthus* extract well were aseptically placed over the seeded agar plates sufficiently separated from each other to avoid overlapping of inhibition zones. The plates were incubated at 37o C for 24 hours and the diameter of the inhibition zones was measured in millimetre.

Antibacterial activity of *Trigonella* extract against bacteria

The antibacterial activity of the crude extract of the *Trigonella* was to be identified against the isolated human pathogenic bacteria. The Well diffusion method was used to find the level of zone formation and the antibacterial activity of the extract.

Antibacterial activity of *Phyllanthus*extract against bacteria

The antibacterial activity of the crude extract of the *Phyllanthus* was to be identified against the isolated human pathogenic bacteria. The Well diffusion method was used to find the level of zone formation and the antibacterial activity of the extract.

The combined antibacterial activity of *Trigonella* and *phyllanthus*

The extracts of the *Trigonella* and the *Phyllanthus* were combined in certain concentrations and the antibacterial activity was analysed by the well diffusion method. This gives the combined effect of the extracts over the pathogens.

Anti-oxidant properties of extract (Vijayasanthi *et al*., 2018)

Determination of total antioxidant capacity (TAC)

Total antioxidant activity of crude *Trigonella* and *Phyllanthus*extract was determined. 7.45 ml sulphuric acid (0.6M), 0.9942g of sodium sulfate (28mM) and 1.235g of ammonium molybdate (4mM) was mixed together in 250ml with distilled water and labelled as total antioxidant capacity. 0.1ml of the EPS extract (200, 400, 600, 800 and 1000µg) was dissolved in 1 ml of total antioxidant capacity and absorbance was read at 695nm after 15min. Ascorbic acid was used as standard.

Scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals

Scavenging of DPPH radicals was measured for the extract. In brief, 1 mL of sample solution at concentrations of 200µl, 400µl, 600µl, 800 µl, 1000 µl ethanol solution of DPPH. Absorbance at 517 nm was measured after 30 min. Scavenging of DPPH radicals was calculated according to: scavenging ability (%) = (1 – A sample/A control) x 100, where A control is the absorbance of control without the tested samples and A sample is the absorbance in the presence of the tested samples. Butylated hydroxyl toluene (BHT) and Vitamin C (Vc) was used as positive controls.

RESULT

Collection of sample

Trigonella

The *Trigonella* sample was collected from Sivakasi local market. 500gm of *Trigonella* seeds were purchased and air dried in room temperature for 2 days. Then the *Trigonella* seeds weregrained almost finely and it weighted upto 50gm for the further process and was given in fig.1

Fig. 1.*Trigonella*

Phyllanthus

The *Phyllanthus*was collected from srivilliputhur local area. 500gm of leaves were collected and air dried in room temperature for 4 days. Then the *Phyllanthus* leaves were grained almost finely and it weighted upto 50gm for the further process and was given in fig.2

Fig. 2.*Phyllanthus*

Preparation of extract

The extracts of *Trigonella* and *Phyllanthus* were prepared using methanol in the sozhelt extraction at 600C for 6hrs. then the extracts were collected and stored for the further analysis and was given in fig.3 and fig.4

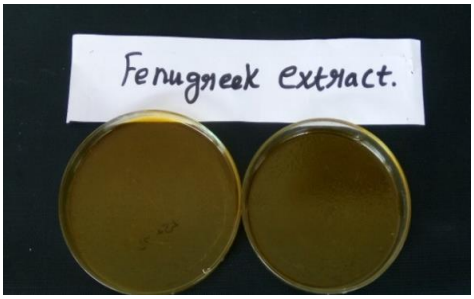

Fig. 3.Methanolic Extract of *Trigonella*

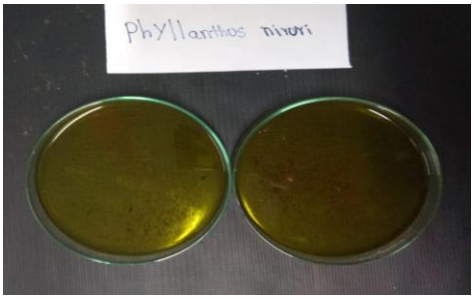

Fig. 4.Methanolic Extract of *Phyllanthus*

Phytochemical screening report from *Trigonella* and *Phyllanthus*

The seed extract of methanol solvent was screen for the presence of variety of bioactive phytochemical compounds. The analysis revealed attendance of bioactive compound such as carbohydrate, protein, amino acids, steroid, phenols, glycosides, flavonoids and alkaloids in methanol extract (Table 1 and 2).

Table 1 Phytochemical screening of *Phyllanthus*

S.No	Name of the compounds	Methanol extract
1	Alkaloids	Present
2	Steroids	Present
3	Glycosides	Present
4	Terpenoids	Present
5	Phenols	Present
6	Tannins	Present
7	Saponins	Present
8	Flavonoids	Present
9	Phlobatanins	Present
10	Quinones	Negative

Table 2. Phytochemical screening of *Trigonella*

S.No.	Name of the compounds	Methanol extract
1	Alkaloids	Present
2	Steroids	Present
3	Glycosides	Present
4	Terpenoids	Present
5	Phenols	Present
6	Tannins	Present
7	Saponins	Present
8	Flavonoids	Present
9	Phlobatanins	Present
10	Quinones	Present

GC- MS report for methanolic extract of *Trigonella*

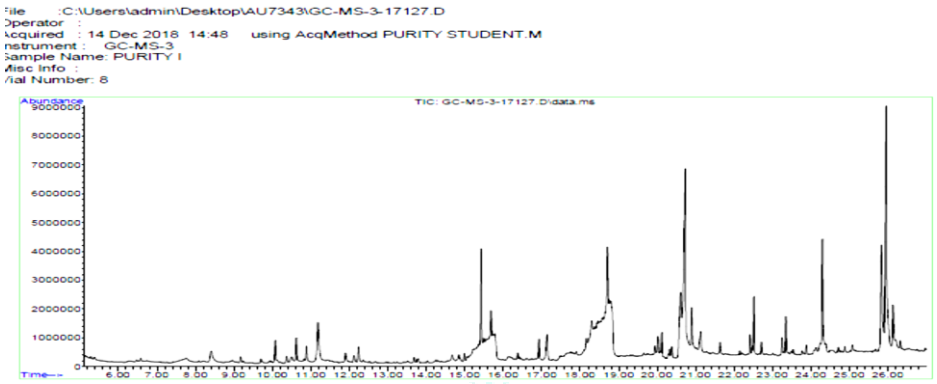

Fig.5. GC- MS report for methanolic extract of *Trigonella*

The compounds said to be present in the *Trigonella*wereErythritol, Methanamine, N-hydroxy-N-methyl-, 1,2-Ethanedithiol, 6,8-Dioxabicyclo[3.2.1]octane, 2-Methyliminoperhydro-1,3-oxazine, 1,4-Butanediamine, 2,3-dimethoxy..., 2-Propen-1-amine N-ethyl-, 2-Methylpyrrolidine, Aziridine, 1,2,3-trimethyl-, trans- 4H-Pyran-4-one, 3,5-dihydroxy-2-, Methylthiouracil, Sarcosine anhydride, Aziridine, 1,2,3-trimethyl-, trans- 1-Methoxy-2,3-cis-dimethylazirid., (R)-(-)-2-Pyrrolidinemethanol, Thiophene, 2,5-difluoro, Benzene, (ethenyloxy)-,1,2,4-Triazolo(4,3-a) pyrimidine 2-Methoxythiophene, 4,5-Dihydro-3-furoic acid, 2-Butenoic acid, 2,3-dimethyl-, 5-Hydroxymethylfurfural, 4-Hexen-3-one, 4,5-dimethyl-, 3-Amino-4,5-dimethyl-2(5H)-furanone, 2,5-Pyrrolidinedione, 1-ethyl- , 2,5-Pyrrolidinedione, 1-ethyl-, 2-Butenoic acid, 2,3-dimethyl-, 2-Methoxythiophene.

GC- MS report for methanolic extract of *Phyllanthus*

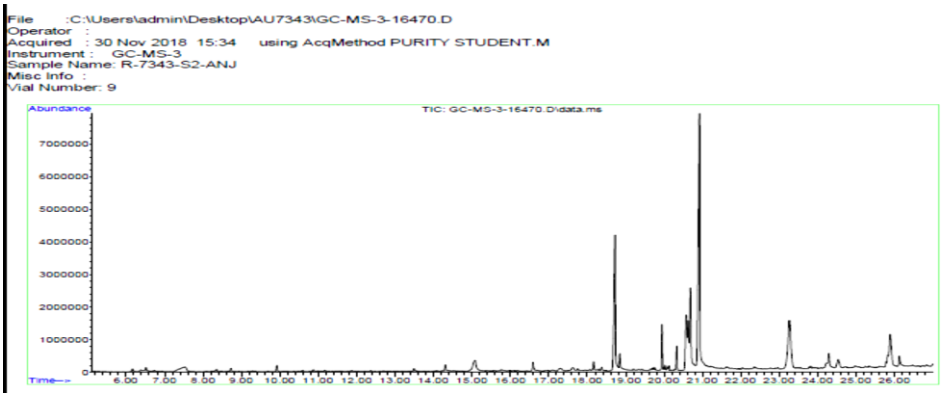

Fig.6.GC- MS for methanolic extract of *Phyllanthus*

The compounds said to be present in the *Phyllanthus*methanolic extraction were Boric acid, Dimethylphosphine, Dimethyl sulfideSilane, trimethylpropoxy-, [(1-Ethoxypropan-2-yl)oxy]trimet., (1-Butoxypropan-2-yloxy)trimethy..., Glycerin, Methanamine, N-methoxy-, Propane, 2-fluoro-2-methyl-,1 1,4-Butanediamine, 2,3-dimethoxy..., Piperazine, 2,5-dimethyl- , Piperazine, 2,5-dimethyl-, trans-,1,3,5-Triazine-2,4,6-triamine Thymine, 4H-Pyran-4-one, 2,3-dihydro-3,5-...,.alpha.-[Di-n-butylaminomethyl], Isopropylimidazole-2-thione, N-Methylpyrrolidine-2,2-dicarbox..., Dodecanoic acid, 1-tert-Butoxypropan-2-yl 2-methy..., 1-(2-Hydroxyethylthio) -2-(2-viny..., Glycine, N-[N-(N-acetyl-L-alanyl..., Tetra decanoicacid, Hexadecanoic acid, methyl ester, Hexadecanoic acid, methyl ester, Pentadecanoic acid, 14-methyl-,2,5-Dihydroxy-4-isopropyl-2,4,6-..., Silane, trimethyl (4-methyl-3-pen..., 6-Amino-2,4-dimethylphenol, n-Hexadecanoic acid, 8-Octadecenal, 1-Tetradecene, 9-Octadecenoic acid (Z)-, methyl..., cis-13-Octadecenoic acid, methyl..., 9,12-Octadecadienoic acid (Z,Z)-..., 9-Octadecenoic acid, (E)- Oleic Acid, 6-Octadecenoic acid.

Isolation of clinical pathogens

Clinical sample collection

The urine and sputum was collected from Sivakasi Government hospital and the samples were serially diluted and plated. The isolated strain were used for the further analysis and was given in fig. 11

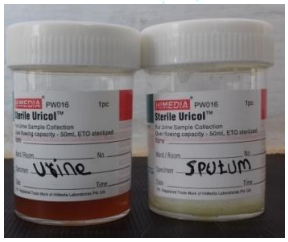

Fig.7. collected urine and sputum sample.

Isolation and Identification of bacteria from clinical samples

The clinical samples were further processed and were incorporated in the respective agar media to isolate the pathogens present in them. The isolated bacteria were named as B1, B2, B3 and B4. These bacteria were further identified microscopically. From observations the isolated organism may be namely *Klebsiella*sp. *Pseudomonas* sp. *Proteus*sp and *Staphylococcus* sp. respectively were isolated and characterized by morphologically and biochemically. Fig.12, table 3 and table 4.

Table 3: Morphological characters of isolates

Isolate	Morphology Characters
B1	Gram negative, rod
B2	Gram positive, rod
B3	Gram negative, rod
B4	Gram positive, cocci

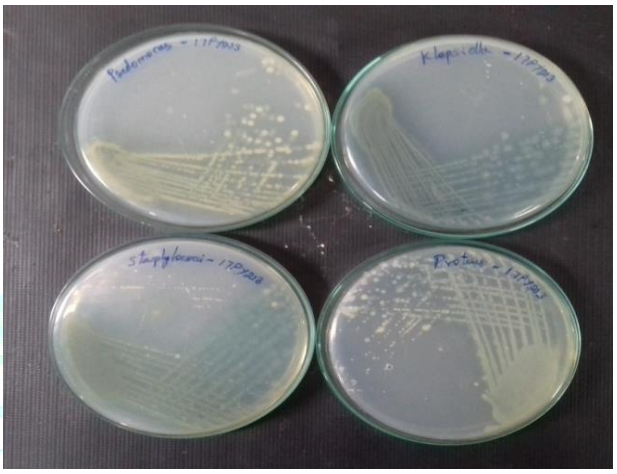

Fig.8. pathogens isolated from clinical sample.

Table. 4 Biochemical characterizations of isolated bacteria

No.of. starins	Indole test	Methyl red test	Vogus proskauer test	Simmon citrate test	Organisms
B1	_	+	_	+	*Klebsiella*
B2	_	_	_	+	*Pseudomonas. Sp.*
B3	+	+	_	+	*Proteus* sp.
B4	_	+	_	+	*Staphylococcus.* Sp.

The *E. coli* culture was directly bought from the SMR diagnostic at Sivakasi. The culture was further used for the work.

Antimicrobial Activity of *Trigonella* and *phyllanthus* Samples

Antibacterial activity of *Trigonella*

The antibacterial activity of *Trigonella* was analyzed. The zone of inhibition was observed for *Staphylococci* about 52mm at 300 µl concentration of the extract and was given in Table 5, Fig 9.

Table. 5 Antibacterial activity of *Trigonella*

Pathogens	Zone in methanol (mm)	Zone in extract (mm)
Klebsiella	2	49
Pseudomonas. Sp.	2	40
Proteus sp.	1	35
Staphylococcus. Sp.	2	52
E coli	2	12

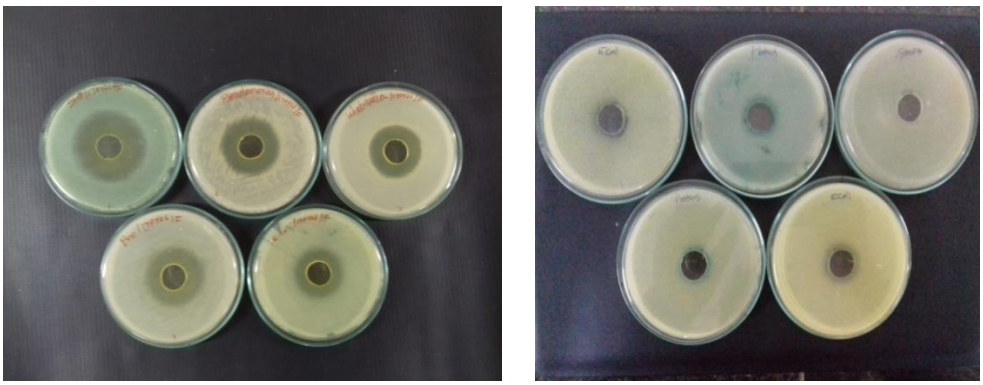

Fig.9 Antibacterial activity of *Trigonella* with control methanol

Antibacterial activity of *Phyllanthus*

The antibacterial activity of *Phyllanthus*was analyzed. The zone of inhibition was observed for *E coli* about 29mm at 300 µl concentration of the extract and was given Table6, fig.10.

Table 6.Antibacterial activity of *Trigonella*

Pathogens	Zone in methanol (mm)	Zone in extract (mm)
Klebsiella	2	22
Pseudomonas. Sp.	2	23
Proteus sp.	1	20
Staphylococcus. Sp.	2	12
E coli	2	29

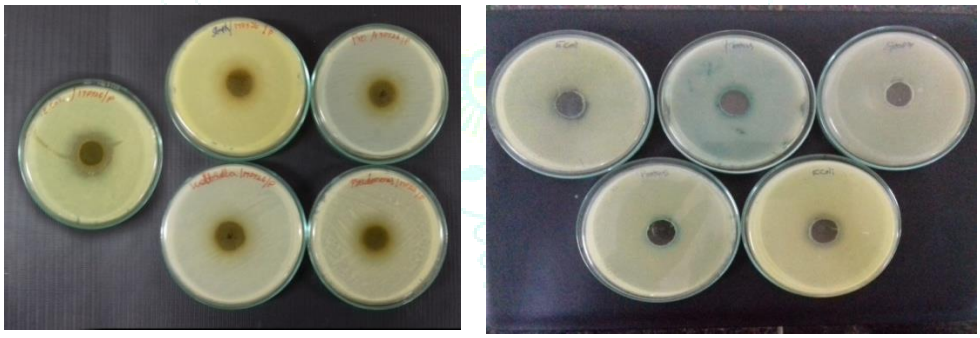

Fig.10 Antibacterial activity of *Phyllanthus* with control methanol

Antibacterial activity of *Trigonella* and *Phyllanthus*

Antibacterial activity of *Trigonella* and *Phyllanthus* was evaluated against human pathogenic bacteria *Staphylococcus* sps, *E. coli*, *Klebsiella*s ps, *Pseudomonas* sps and *Proteus* sp. In first screening of effectivity of *Trigonella* and *Phyllanthus* fraction on clinical pathogenic microoraganism was performed by Muller Hinton agar well diffusion methods. The *Trigonella* and *Phyllanthus* extract used for antibacterial effectiveness show significant activity. It has been observed that methanol extract of *Trigonella* and *Phyllanthus* were effective against clinical pathogenic microoraganism (fig.11and table 7)

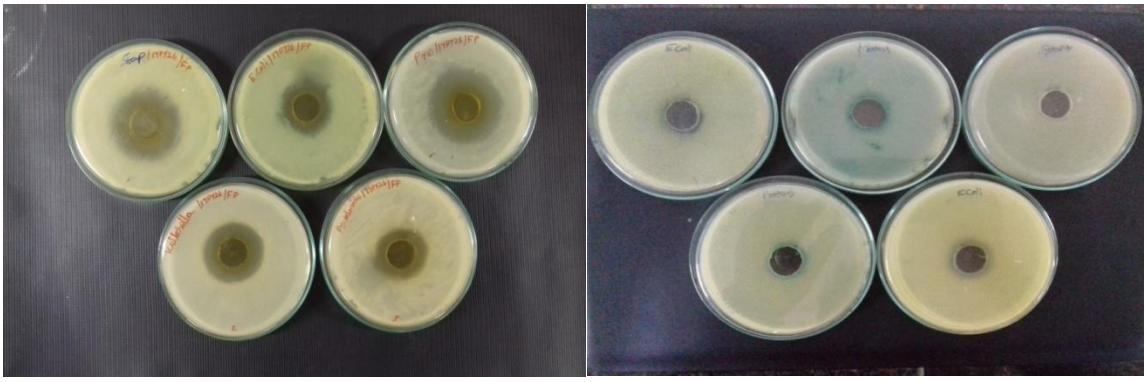

Fig. 11.Antibacterial activity of *Trigonella* and *Phyllanthus* with methanol control

Table 7 Antibacterial activity of *Trigonella* and *Phyllanthus*

S.No.	Microorganism	Control	*Trigonella* Extract	*Phyllanthus*extract	*Trigonella* and *Phyllanthus*
1	*pseudomonas*	_	40 mm	_	31mm
2	*klebsiella*	_	38 mm	_	36mm
3	*E.coli*	_	31 mm	29 mm	30mm
4	*staphyllococcus*	_	52 mm	_	45mm
5	*proteus*	_	36 mm	_	48mm

Antioxidant activity of extracts

Total antioxidant capacity of *Trigonella*and *Phyllanthus*

The anti-oxidant activity of the *Trigonella* was analyzed for the extract. The total antioxidant capacity was performed for the extract. The total antioxidant capacity was found to be 1.26 µg/ ml and was given in fig.13.

The anti-oxidant activity of the *Phyllanthus* was analyzed for the extract. The total antioxidant capacity was performed for the extract. The total antioxidant capacity was found to be 1.03 µg/ ml and was given in fig.14.

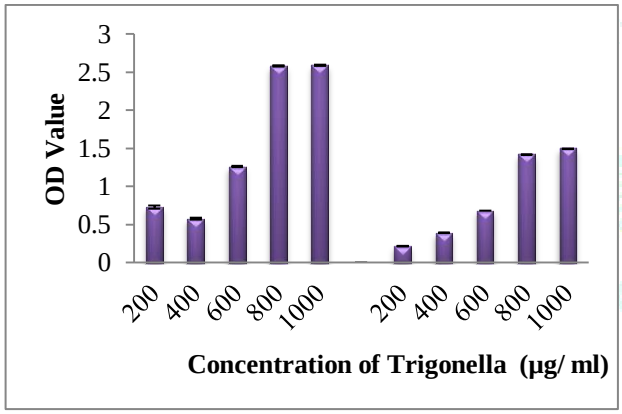

Fig.13 Total antioxidant capacity of *Trigonella*

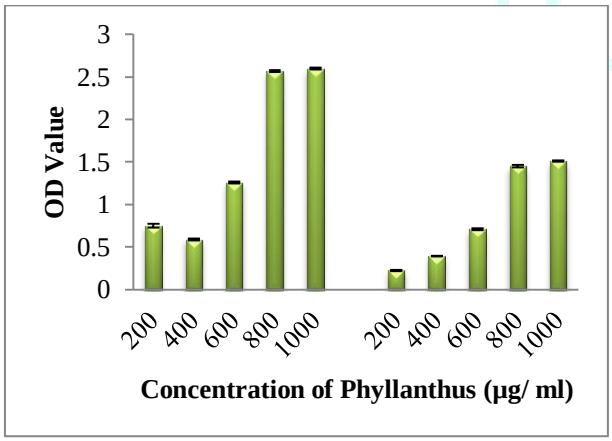

Fig.14 Total antioxidant capacity of *Phyllanthus*

DPPH assay for *Trigonella*and *Phyllanthus*

The DPPH assay was performed for the *Trigonella*extract and the percentage of inhibition was found to be 85.9 + 0.2 and was given in fig.15. The DPPH assay was performed for the *Phyllanthus*extract and the percentage of inhibition was found to be 85.9 + 0.2 and was given fig.16.

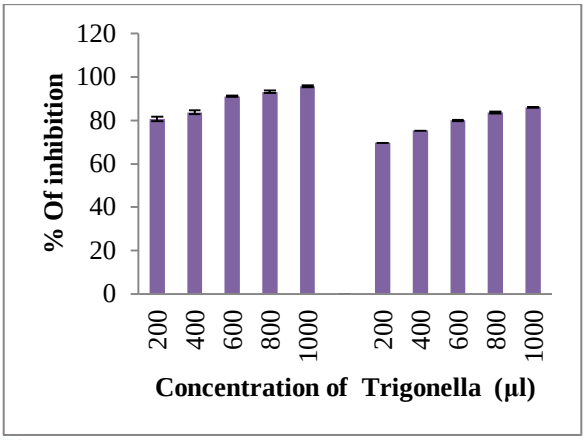

Fig. 15 DPPH assay for *Trigonella*

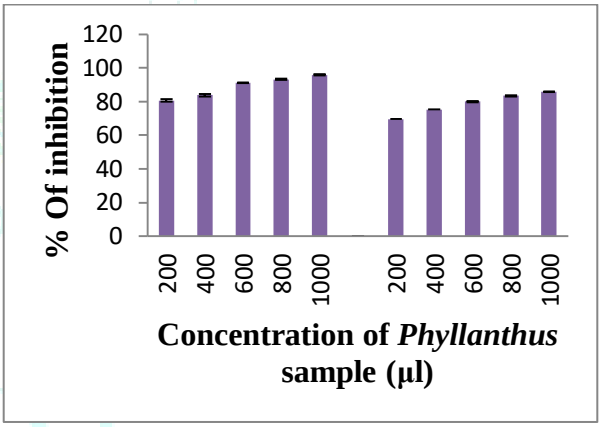

Fig. 16. DPPH assay for *Phyllanthus*

DISCUSSION

In the present study the methanolic extracts of *Trigonella* and *Phyllanthus* were prepared by the soxhelt extraction. The extracts were further dried to analyze the antimicrobial activity against certain clinical pathogens.

Similarly Renu *et al.* (2016) reported that extracts of *Trigonella* and *Phyllanthus* were obtained using methanol. The extracts were used for the various analysis and characterization studies. It was also reported that methanolic extract showed higher activity to restrict the growth of bacterial pathogens.

In the present study the seed and leaf extract of *Trigonella* and *Phyllanthus* were screened for the presence of bioactive phytochemical compounds respectively. Thus, the phytochemical compounds present in the *Trigonella* and *Phyllanthus* were tannins, anthraquinones, flavonoids, alkaloids, terpenoids, saponins, cardiac glycosides, reducing sugars, phlobatanins, steroids, amino acids, phenolic and proteins respectively.In the same way Sita *et al. (*2016) reported that the methanolic extract of *Trigonella* seeds and *Phyllanthus* leaves consists of terpenoids, saponins, cardiac glycosides, reducing sugars,

phlobatanins, steroids, tannins, anthraquinones, flavonoids, alkaloids amino acids, phenolic and protein.

Moradi*et al.* (2013) detailed that *Trigonella* is a characteristic well source of iron, silicon, sodium and thiamine. *Trigonella* contains mucilagins which are known for alleviating and relaxing of kindled tissues. *Trigonella* seeds contain alkaloids, including trigonelline, gentianine and carpine compounds, 4-hydroyisoleucine, 1-Methoxy-2,3-cis-dimethylazirid, 6,8-Dioxabicyclo[3.2.1]octane and *Trigonella*ine, and a component which have medical property of hypoglycemic activity. The mechanism of phytochemicals in the *Trigonella* may also increase the number of insulin receptors in red blood cells and improve glucose utilization in peripheral tissues, thus demonstrating potential anti-diabetic effects both on the pancreas and other sites. It was reported that the compound amino acid 4-hydroxyisoleucine, present in seeds, directly stimulate insulin secretion in humans.

In the present study it was reported that the *Trigonella* methanolic extraction which was fine for the GC-MS analyses have several compounds were Erythritol, Methanamine, N-hydroxy-N-methyl-, 1,2-Ethanedithiol, 6,8-Dioxabicyclo[3.2.1]octane, 2-Methyl iminoperhydro-1,3-oxazine, 1,4-Butanediamine, 2,3-dimethoxy..., 2-Propen-1-amine N-ethyl-, 2-Methylpyrrolidine , Aziridine, 1,2,3-trimethyl-, trans- 4H-Pyran-4-one, 3,5-dihydroxy-2-... , Methyl thiouracil , Sarcosine anhydride, Aziridine, 1,2,3-trimethyl-, trans- , 1-Methoxy-2,3-cis-dimethylazirid..., (R)-(-)-2-Pyrrolidinemethanol, thiophene, 2,5-difluoro were found to be present. These were the phytochemicals present in the methanolic extract of *Trigonella*.

In the present study it was reported that GC-MS report of extract of *Phyllanthus* prepared through methanol was found to have several compounds. The compounds present in the extract was Boric acid, Dimethyl phosphine, Dimethyl sulfideSilane, trimethylpropoxy-, [(1-Ethoxypropan-2-yl)oxy] trimet, (1-Butoxypropan-2-yloxy) trimethy..., Glycerin, Methanamine, N-methoxy-Propane, 2-fluoro-2-methyl-1 1,4-Butanediamine, 2,3-dimethoxy..., Piperazine, 2,5-dimethyl-, Piperazine, 2,5-dimethyl-, trans-,1,3,5-Triazine-2,4,6-triamine Thymine, 4H-Pyran-4-one, 2,3-dihydro-3,5-..., alpha.-[Di-n-butylaminomethyl] were compound present. These compounds might be having the antifungal and antibacterial activity.

Similarly kaviarasan*et al.* (2018) reported that GC-MS report of methanolic extracts of *Phyllanthus*niruricontains 1-o-galloyl-6-o-luteoyl-a-Dglucose that showed growth reduction against *Plasmodium falciparum*. The compounds glucogallin, quercetin 3-o-b-Dglucopyranosyl-(2 to 1)-o-b-D- xylopyranoside, 1 1,4-Butanediamine, Propane, 2-fluoro-2-methyl, b-sitosterol and gallic acid were isolated.

In the present study the clinical pathogens which are said to nasocomial was isolated from the sputum and urine samples of patients. The nutrient agar media is used of obtains the colonies which are the suspected to be the clinical pathogenic microorganism presented. Identification was carried out on the bases of morphological and biochemical characteristic through gram staining for the clinical pathogens. The isolated bacteria were further identified by the respective media. Thus *Klebsiella*sp, *Pseudomonas* sp, *Staphylococcus* sp, *Proteus*sp and *E. coli* was directly obtained from the clinical laboratory.

Similarly Srinivasan, (2015) reports that bacteria were isolated from clinical samples. Selective media such as Mac Conkey, EMB and Mannitol salt agar media were used for the isolation of *E. coli* and *Staphylococcus* sp. Identification was carried out on the basis of morphological and biochemical characteristic through gram staining, catalase test, indole production test, methyl red test, voges-proskauer test, starch hydrolysis, sugar fermentation.

In the presented study it was observed that the highest antibacterial activity was seen with methanol extract of *Trigonella* and *Phyllanthus* which may be due to the presence of certain phytochemicals present in the methanolic extracts. Methanol extract give the fine result for the further analysis compared with other extracts, aqueous solvent, ethanol solvent and ethyl acetate solvent.

In the present study it was observed that the *Trigonella* extraction of the isolated microorganisms antibacterial activity. Thus it showed zone of inhibition for *Klebsiella*sp, *Pseudomonas*sp, *Staphylococcus*sp, *Proteus*sp and *E. coli*. It showed zone inhibition for human pathogenic bacteria of about *Staphylococci* of 52mm of zone and *Phyllanthus* extract showed 29mm of zone for *E coli*.

Similarly Kumar *et al.* (2009) reported that Gram positive and Gram negative bacterial and fungal strains are used to examine the anti-microbial activity of *Trigonella* seed crude extract. Antimicrobial activity is identified by measuring the zone of inhibition. The antimicrobial activity was processed by agar well diffusion method by using different concentrations 2.5,5.0,7.0,10µg/ml and the zone of inhibition was observed for the extracts.

In the present study the total antioxidant capacity of the *Trigonella* and *Phyllanthus* was 1.26 and 1.03 µg/ml. The DPPH assay reveals the free radical scavenging activity. The free radical sacvenging activity of *Trigonella* was 85.9 + 0.2 and for *Phyllanthus* was 85.02 + 0.2 respectively.

Similarly Gafner*et al.,* (2016) reported that total antioxidant capacity of the methanolic*Trigonella* was found to be 3.96 µg/ml. The DPPH assay revealed 83 + 0.5 of free radical scavenging activity. The *Phyllanthus* 5.12 µg/ml showed of total antioxidant capacity. The DPPH assay of the *Phyllanthus* extracted with methanol showed about 89 + 0.80.

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