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Analysis of Antioxidant, Antimicrobial Activity and *Phytochemical Potential* of *Cleistanthus collinus Roxb.*, Polygonum *glabrum* Wild. and *Melia azedarch* Linn.

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Abstract

Plants having potential phytochemical constituents which are capable of curing various human ailments and prevent disorders. Among the phytochemical constituents, antioxidant and antimicrobial bioactive compounds are playing a key role in society especially in health care Industry. Based on the available scientific information, the present study is planned to collect the plant material of *Cleistanthus collinus*, *Polygonam glabrum* and *Melia azedarch* from Jawadhu hills for the analysis of phytochemical potential including antioxidant and antimicrobial activity. The bioactive compounds present in the plant samples were extracted with solvents like methanol, ethyl acetate, chloroform and ether and analysed by standard procedures. The results of the present study show that the antioxidant potential of the all the plant extracts were found to be potent and remarkable radical scavenging activity. The IC_{50} values were recorded as 64.54, 79.81 and 75.0 µg/ml for *C. collinus*, *P. glabrum* and *M. azedarch*, respectively. The antibacterial activity of the methanol extracts of *C. collinus* exhibited maximum activity against all the tested pathogens such as *S. aureus*, *M. luteus*, *E. coli* and *P. aeruginosa*, with a maximum Zone of inhibition (ZOI) of 15, 11, 12 and 18 mm respectively. The antifungal potential of the plant extracts was found to be less significant, as only *C. collinus* showed inhibition of 11 mm against both *C. albicans* and *C. tropicali.* The phytochemical analysis revealed the presence of compounds like Flavonoids, phenols, tannins, terpenoids and reducing sugars in all the above plants at significant level. Thus these plants could be explored further for a significant source of bioactive compounds.

Keywords: Phyto-chemical analysis, Antioxidant activity, Antimicrobial activity, *Polygonam glabrum* and *Melia azedarch*, *Cleistanthus collinus*,

Contents

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1. Introduction

Over the years, the World Health Organization advocated that countries should interact with traditional medicine with a view to identifying and exploiting aspects that provide safe and effective remedies for ailments of both microbial and non-microbial origins (Akinyemi *et al*, 2005). Medicinal plants constitute an effective source of both traditional and modern medicines. The development of bacterial resistance to presently available antibiotics has necessitated the need to search for new antibacterial agents. Gram positive bacteria such as *Staphylococcus aureus*a remainly responsible for post-operative wound infections, toxic shock syndrome, endocarditis, osteomyelitis and food poisoning (Beneyache *et al*., 2001). Gram negative bacterium such as *Escherichia coli* is present in human intestine and causes lower urinary tract infection, coleocystis or septicaemia. Different antibiotics exercise their inhibitory activity on different pathogenic organisms (Chanda and Rakholiya, 2011).

Multiple drug resistance in human pathogenic microorganisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics, the usage of antibacterial agent, host characteristics and environmental factors. This situation has forced scientists to search for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents, but the cost production of synthetic drugs is high and they produce adverse effects compared to plant derived drugs (Abiramasundari *et al*., 2011). Phytochemical screening of the crude extracts of stems, leaves and flowers revealed the presence of different kind of chemical groups such as flavonoids, tannins, alkaloids, saponins and carbohydrates. The amounts of total phenolics solvent extracts (methanol and water extract) for the three parts of plant were determined spectrometrically. (Meryem Seladji et *al*., 2014). From this study it is obvious that C. collinus leaf extracts contains many biologically active compounds in various concentrations and also it gives a detailed insight about the phytochemical profile which could be exploited for the development of plant based drug. (Suman *et al.,* 2013).

These antimicrobial substances are of natural origin, and it is thought that their influences on the environment are few and can be used as biological control agents. However, some medicinal herbs for some reasons have not found wider application and sometimes are referred as 'forgotten plants'. Taking into account the increasing demand for natural ingredients that might be used as food additives, components of functional foods, preventing plant diseases and nutraceuticals as well as for other applications. It is reasonable to revise the 'forgotten plants' by assessing their applicability and benefits using modern scientific analysis methods (Abdel Rahman *et al*., 2011).

Organic crude extracts from both fresh and dry leaves could be used as potential sources of new antioxidant and antimicrobial agents (Tahiya Hilal Ali Alabri *et al*., 2013). Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. Phytochemical Analysis revealed the presence of alkaloids, flavonoids, saponins, phenols, steroids, Anthraquinones and tannins. (Govindappa *et al*., 2011). The plant indicated that the methanolic extract of the leaves are pharmacologically more active than the other extracts (Hegde Chaitra R. *et al*., 2012). Such properties may be of great use in mitigating the detrimental effects of oxidative stress and reducing susceptibility to bacterial infection. Notably, extracts of E. kologa leaf also contain proteins and carbohydrates which add to its nutritional value.(Zoe Merculieff *et al.,* 2014). Therefore, based on the above informations, the present study is focused to analyse the palnt species as *Cleistanthus collinus* Roxb., *Polygonum glabrum* Wild. and *Melia azedarach* Linn. collected from Javadhu hills of Tamil Nadu. For the presence of antioxidant and antimicrobial potential and overall phyotchemical profile to know the significance.

2. Materials and Method

General laboratory techniques recommended by Purvis *et al.,* (1966) was followed for the preparation of media, inoculation and maintenance of cultures.

Chemicals and glassware: All the glassware (Borosil, Anumbra or Corning) were immersed in cleaning solution for 2 to 3 hours and washed thoroughly with tap water followed by detergent solution and finally rinsed with distilled water. The cleaned glassware were dried in hot air oven and stored for future use. The cleaning solution was prepared using potassium dichromate with sulphuric acid after the formulation suggested by Mahadevan and Sridhar, 1996**.** Dried glassware was sterilized by using hot air oven or with an autoclave for 15 min at 15 lb/sq inch pressure. All the chemicals used in the present study were analytical grade.

Plant collection: Fresh leaves of *Cleistanthus collinus* Roxb., *Polygonum glabrum* Wild. and *Melia azedarach* Linn., were collected from the fields located in Jawadhu Hills, Thiruvannamalai District, Tamil Nadu.

The habit and morphological appearance of the above plant species were given in **Fig. 1**

***Cleistanthus collinus* Roxb.**

***Polygonum glabrum* Wild.**

***Melia azedarach* Linn.**

Figure 1: Morphology of plant species selected in the present study

Culture media and Maintenance of microbial culture

General laboratory techniques recommended by Purvis *et al.*, (1966) was followed for the preparation of media, culturing and maintenance of microbial cultures. Different media were used in this work for different purposes such as screening and dye assays. For isolation, subculture and maintenance of both bacterial and fungal cultures, Nutrient agar (NA), Nutrient broth and Potato dextrose agar (PDA) of slants and in petriplates were used. Standard cultures of Bacteria and fungi were used in this study for antimicrobial test.

Preparation of crude extracts: Extraction of leaf powder with methanol was performed by direct method as given by Eloff, (1998). In this method, finely ground plant material was extracted with chloroform, ethyl acetate and methanol in the ratio of 1:10 in conical flask in shaking condition for overnight. The extract was filtered through the Whatmann No. 1 filter paper in a separate container. The above process was repeated 3 times and the same plant material but using fresh solvent. The solvent was removed by placing the extracts in distillation unit in the respective temperature. The extracted residues were weighed and re-dissolved in different solvents to yield 10 mg/ml solutions ready for further analysis.

Antibacterial activity–Disc diffusion assay (Eloff, 1998): Nutrient agar was prepared and poured in the sterile Petri dishes and allowed to solidify 24 hr growing bacterial cultures *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli*, *Pseudomonas aeruginosa* were swabbed on it. Sterile filter paper discs (9mm) were placed on the plates. Four different concentrations (250µg, 500µg, 750µg and 1000µg) of the plant extracts and one negative control (DMSO) were loaded on the discs and placed inverted on the plates. The plates were then incubated at 37ºC for 24h. After incubation the zone of inhibition was measured.

Antifungal activity-Well diffusion assay (Eloff, 1998): Potato dextrose agar was prepared and poured in the sterile Petri dishes and allowed to solidify. The 24 h growing fungal cultures *C. albicans* and *C. tropicalis* were swabbed on it. Sterile filter paper discs (9mm) were placed on the plates. Four different concentrations (250µg, 500µg, 750µg and 1000µg) of the plant extracts and one negative control (DMSO) were loaded on the discs and placed inverted on the plates. The plates were then incubated at 37ºC for 24h. After incubation the zone of inhibition was measured.

Evaluation of antioxidant potential-DPPH assay: **(2, 2-diphenyl-1-picrylhydrazyl):** The Radical Scavenging Activity of different extracts was determined by using DPPH assay according to Chang *et al.*, (2008) with small modification. The decrease of the absorption at 517nm of the DPPH solution after the addition of the antioxidant was measured in a cuvette containing 2.96ml of 0.1 m Methanolic DPPH solution mixed with 40 µl of coconut apple

extract (20 to 200µg/ml) and vortexed thoroughly. The setup was left at dark in room temperature and the absorption was measured at 517nm after 20 minutes. The ability of the plant extract to scavenge DPPH radical was calculated by the following equation:

$$\% \text{ of DPPH Radical Scavenging Activity (\% RSA)} = \frac{\text{Abs. control} - \text{Abs. sample} * 100}{\text{Abs. control}}$$

Abs. control is the absorbance of DPPH radical + ethanol;
Abs. sample is the absorbance of DPPH radical + plant extract.

Qualitative Phytochemical Screening

Test for Alkaloids by Mayer's Test:

Solvent free extract (50mg) was stirred with 2mL of dilute hydrochloric acid (1mL HCL + 1mL water) and filtered. The filtrate was tested carefully with various alkaloid reagents. To 2mL of filtrate, a drop or two of Mayers's reagent was added by the sides of the test tube. A white creamy precipitate indicated the presence of alkaloids (Evans, 1997). To prepare Mayer's reagent, Mercuric chloride (0.135g) was dissolved in 6ml of water and potassium chloride (0.5g) was dissolved in 1mL of water. The two solutions were mixed and made up to 10mL with water.

Test for Phenolic Compounds by Ferric Chloride Test: The extract (50mg) was dissolved in 1 mL of distilled water. To this few drops of neutral 5% ferric chloride solution was added. A dark green color indicated the presence of phenol (Mace, 1963). To prepare ferric chloride solution, about 0.5g of ferric chloride was dissolved in 10mL of distilled water.

Test for Glycosides by Borntrager's Test:

About 50mg of extract was hydrolysed with 5mL of concentrated hydrochloric acid for two hours on a water bath and filtered. To 2mL of filtrate hydrosylate, 3mL of chloroform was added and shaken, chloroform layer was separated and 10% ammonia solution was added to it. Appearance of a pink, red or violet colour in the ammoniacal (lower) phase indicated the presence of glycosides (Evans, 1997).

Test for Terpenoids by Salkowski Test: About 0.5g of the extract was added in 2mL of chloroform and concentrated sulphuric acid (3ml) and was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids (Sofowora, 1993).

Test for Flavonoids by Sodium Hydroxide Test:

About 0.5g of extract was dissolved in 5mL of distilled water and filtered. To 2mL of filtrate small quantity of the each portion was dissolved in water and filtered and to this, 2 ml of the 10% aqueous sodium hydroxide was later added to produce a yellow colouration. A change in colour from yellow to colorless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids (Trease and Evans, 2002).

Test for Tannins by Neutral Ferric Chloride: About 0.5g of the extract was boiled in 10mL of water in test tube and then filtered. A few drops of 1% ferric chloride was added and observed for blue-green, green or brownish green precipitate indicates the presence of tannins (Trease and Evans, 2002). About 0.01g of ferric a chloride was dissolved in 10mL of distilled water for ferric chloride solution.

Test for Reducing Sugars by Fehling's Test: The extract (100mg) was dissolved in 5mL of water and filtered.1mL of filtrate was boiled on water bath with 1mL each of Fehling's solution I and II. A red precipitate indicates the presence of sugars (Trease and Evans, 2002).

Test for Saponin by Foam Test: The extract (50mg) was diluted with 5mL of distilled water .The suspension was shaken in a graduated cylinder for 15mins. A 2cm layer thick of foam indicates the presence of saponins (Kokate, 1999).

Test for Proteins by Biuret Test:

The extract (100mg) was dissolved in 10mL of distilled water and filtered through Whatmann No.1 filter paper and the filtrate was collected. To 2mL of filtrate, one drop 2% of copper sulphate solution.1mL of ethanol(95%) was added, followed by excess of potassium hydroxide pellets(1pellet). Pink color in the ethanolic layer indicates the presence of proteins (Kokate, 1999). To prepare copper sulphate solution, 0.2g of copper sulphate was dissolved in 10mL of distilled water.

Quantitative Phytochemical Analysis

Determination of Phenolic Compounds by Folin Ciocaltaeu's Method: The total phenol content of the extract was measured at 765 nm with Folin Ciocalteu reagent. The dilute methanol extract (0.5 ml of 1:10 g ml-1) or gallic acid (standard phenolic compound) was mixed with 5ml of Folin-Ciocalteu reagent (1:10 diluted with distilled water) and 4ml of aqueous sodium carbonate (1 M). The mixture was allowed to stand for 15 min and the total phenols were determined by spectrophotometer at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg solutions of gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg per gm of dry mass), which is a common reference compound (McDonald *et al.*, 2001).

Determination of Flavonoids by Aluminium Chloride Test:

To 1ml of varying concentrations of extract, 3 ml of methanol, 0.2 ml of 1 M potassium acetate, 0.2 ml of 10% aluminium chloride and 5.6ml of distilled water was added and left at room temperature for 30 minutes. Absorbance of the mixture was read at 415 nm using UV spectrophotometer. Calibration curve was prepared using Quercetin as standard (Yadav*et al.*, 2011).

Thin Layer Chromatography:

A pre-coated silica gel TLC plate approximately 1.5 cm wide and 5 cm long was used in the study. Marked the TLC plate using a pencil about 1cm from the bottom edge and a small spot of solution containing the sample is applied to a plate. The sample is allowed to completely evaporate for better separation A small amount of an appropriate solvent (eluent) poured in to a glass beaker or any other suitable transparent container (separation chamber) to a depth of less than 1 centimeter. The chamber is covered with glass lid and is left for 10 minutes for saturated. The TLC plate is then placed in the chamber so that the spot(s) of the sample do not touch the surface of the eluent in the chamber, and the lid is closed. The chromatogram is allowed to run by capillary action and the sample mixture carries to top of the plate (elutes the sample). When the solvent front reaches no higher than the sample line, then the plate removed and dried (Hao *et al.*, 2004).

3. Results and Discussion

Extraction of plant material

The powdered samples of plant materials were extracted with chloroform, ethyl acetate and methanol and the extracts were stored in a desiccators for further use.

Evaluation of antibacterial efficacy of the plant extracts

The results of the antibacterial assay against *Staphylococcus aureus* show that among 3 plant extracts with 3 different solvent systems, the methanol extract of *C. collinus* possessed significant antibacterial activity (Table 1 and Figure 2). It showed inhibition against all the organisms from the concentration of 50 µg/ml. The ZOI was recorded to vary in the range of 7-15 mm while the maximum inhibition of 15mm was observed at a concentration of 200µg/ml. However, *P. glabrum* and *M. azedarch* possessed similar anti-staphylococcal activity where the inhibition was in the range of 6-11mm with a maximum zone (11mm) recorded for the concentration of 200µg/ml (Table 1).

Table 1: Inhibitory effect of the methanol extracts individual plants on *S. aureus*

S.NO	Plant	Zone of inhibition (mm)			
		50	100	150	200
1	*C. collinus*	7	10	13	15
2	*P. glabrum*	6	7	9	11
3	*M. azedarch*	6	7	8	11

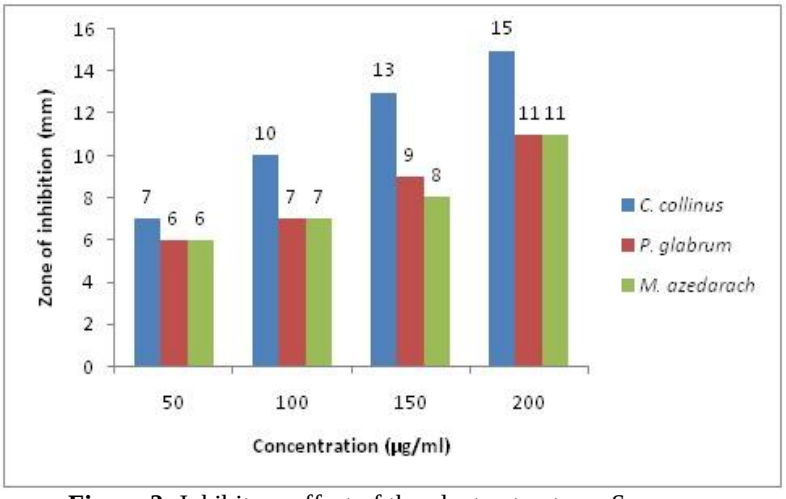

Figure 2: Inhibitory effect of the plant extracts on *S. aureus*

In case of *Micrococcus luteus*, the rate of inhibition was studied in the order of *C. collinus*, *P. glabrum* and *M. azedarch* (Figure 3). The maximum inhibition was exhibited by *C. collinus M. azedarch* with a ZOI of 11mm at a concentration of 200µg/ml (Table 2). However the other two extracts also showed moderate inhibition against *M. luteus.*

Table 2: Inhibitory effect of the plant extracts on *M. luteus*

S.No	Plant	Zone of inhibition (mm)			
		50	100	150	200
1	*C. collinus*	7	8	10	11
2	*P. glabrum*	6	6	6	10
3	*M. azedarch*	6	7	10	11

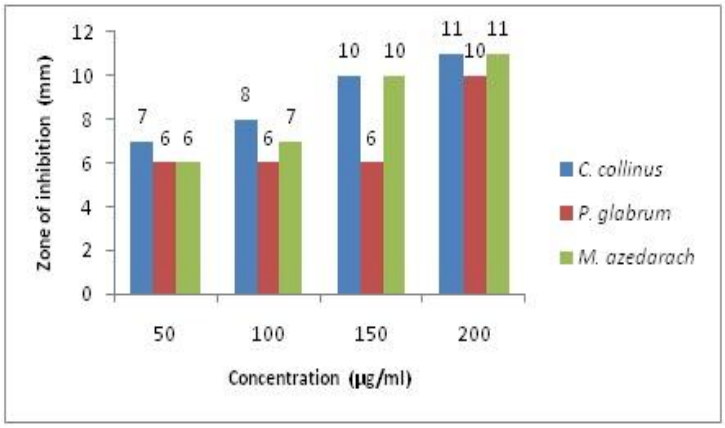

Figure 3: Inhibitory effect of the plant extracts on *M. luteus*

For study on *E. coli*, the zone of inhibition of the methanol extracts of *C. collinus*, *P. glabrum* and *M. azedarch*, which varied in the range of 9-12mm, 6-10mm and 6-12mm, respectively (Table 3 and Fig. 4). The maximum and minimum inhibition was recorded by *C. collinus* and *P. glabrum*, respectively.

Table 3: Inhibitory effect of the plant extracts on *E. coli*

S.No	Plant	Zone of inhibition (mm)			
		50	100	150	200
1	*C. collinus*	9	10	11	12
2	*P. glabrum*	6	7	8	10
3	*M. azedarch*	6	10	12	12

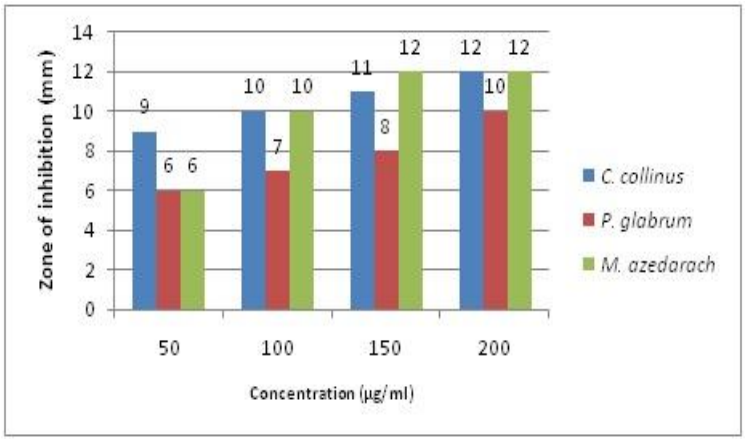

Figure 4: Inhibitory effect of the plant extracts on *E. coli*

Table 4: Inhibitory effect of the plant extracts on *P. aeruginosa*

S.No	Plant	Zone of inhibition (mm)			
		50	100	150	200
1	*C. collinus*	14	15	16	18
2	*P. glabrum*	6	6	8	11
3	*M. azedarch*	6	7	7	10

Furthermore, significant inhibition was recorded against *P. aeruginosa* with a maximum ZOI value of 18 mm by *C. collinus* extract at a concentration of 200µg/ml (**Table 4**). As in earlier cases, *P. aeruginosa* was also least inhibited by *P. glabrum and M. azedarch* (**figure 5**).

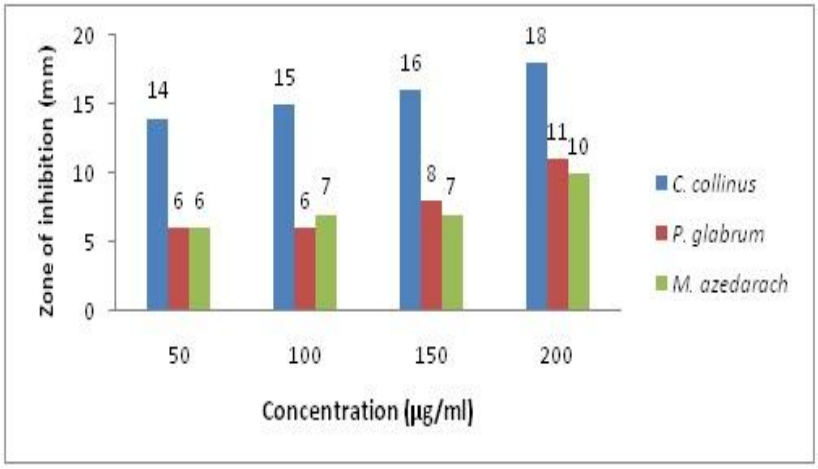

Figure 5: Inhibitory effect of the plant extracts on *P. aeruginosa*

On the whole, the methanol extract of *C. collinus* exhibited maximum inhibition against all the tested pathogens when compared to the other plants.

Evaluation of antifungal property of the extracts

The antifungal activity of the extracts was tested against *C. albicans* and *C. tropicalis*. The results of the assay reveal that only *C. collinus* extract was capable of inhibiting the fungal species tested with a maximum zone of inhibition of 10mm against *C. albicans* and *C. tropicalis* at a concentration of 200µg/ml (**Table 5**). Furthermore, the extracts of *P. glabrum* and *M. azedarch* did not show any significant inhibition in the concentration range tested. The minimum concentration at which the *C. collinus* extract showed inhibition was 100µg/ml for both the test pathogens.

Table 5: Inhibitory effect of the plant extracts on fungal pathogens

S.No	Plant	Zone of inhibition (mm)							
		50		100		150		200	
		C.a	*C.t*	*C.a*	*C. t*	*C. a*	*C. t*	*C. a*	*C. t*
1	*C. collinus*	-	-	6	6	6	6	10	10
2	*P. glabrum*	-	-	-	-	-	-	-	-
3	*M. azedarch*	-	-	-	-	-	-	-	-

Evaluation of antioxidant potential of the plant extracts

The antioxidant property of the plant extracts was studied by DPPH assay. The results of the assay suggest that the methanol extract of *C. collinus* showed better Radical Scavenging Activity (RSA) when compared to *P. glabrum* and *M. azedarch* (Table 6 and Fig. 6). The RSA values of *C.collinus* was recorded in the range of 35.6% - 77.4%, while that for *P. glabrum* and *M. azedarch*, it was found to be in the range of 28.6% - 62.6% and 31.4% - 66.6%, respectively (Table 6).

Table 6: RSA effect of the plant extracts

S.No	Concentration (mg/ml)	RSA (%)		
		C. collinus	*P. glabrum*	*M. azedarch*
1	20	35.632	28.62	31.419
2	40	44.137	38.045	43.793
3	60	54.827	44.942	53.687
4	80	65.287	54.482	60.574
5	100	77.471	62.643	66.666

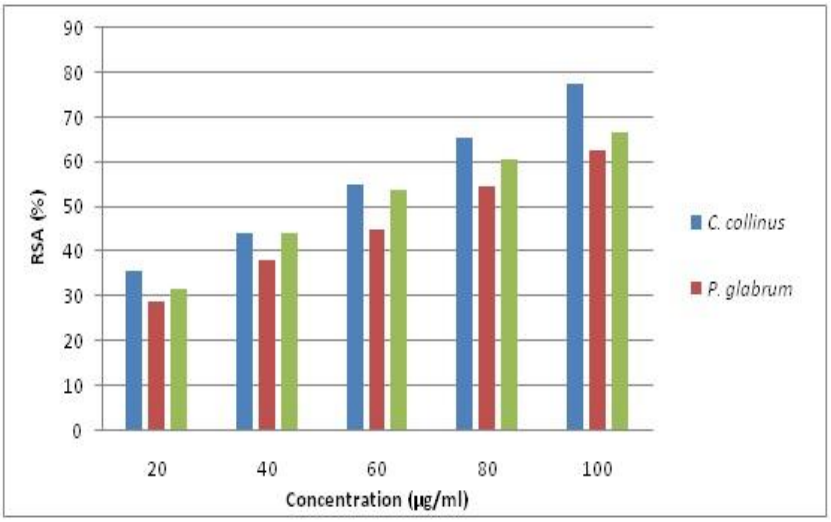

Figure 6: RSA effect of the plant extracts

Qualitative phytochemical screening of the plant extracts

The phytochemical profile of the methanol extracts of the 3 selected plants were studied by various qualitative assays. The data reveals that the tannins, flavonoids and reducing sugars were present in all the 3 extracts. However alkaloids, saponins and proteins were not detected in any of the extracts. In addition, *C. collinus* and *P. glabrum* were found to contain moderate amounts of phenols; *P. glabrum* and *M. azedarch* were found to contain trace amounts of reducing sugars (Table 7 and Fig.7).

Table 7: Qualitative phytochemical screening of the plant extracts

S. No	Phytochemicals	*C. collinus*	*P. glabrum*	*M. azedarch*
1	Aalkaloids	-	-	-
2	Phenolic compound	++ 185.5	+ 20.5	+ 25.0
3	Glycosides	-	-	-
4	Terpenoids	+	+	+
5	Flavonoids	++ 650.8	++ 540.0	+ 200.0
6	Tannins	+++	+	++
7	Reducing sugars	-	+	++
8	Saponins	-	-	-
9	Proteins	-	-	-

- Presence not detected
+ Present in minor amount
++ Present in moderate amount
+++ Present in major amount

Quantitative estimation of phytochemicals

The major phytochemicals such as phenols and flavonoids were further quantified using standard techniques. The amount of total phenols present in the extracts of *C. collinus, P. glabrum* and *M. azedarch* was recorded as, 185.5, 20.5 and 25.0 GAE/g sample. Similarly, the amount of total flavonoids in the *C. collinus, P. glabrum* and *M. azedarch* extracts were found to be 650.8, 540.0 and 200.0 QE/g sample.

Thin Layer Chromatography

The TLC profile of the plant extracts revealed the presence of 11, 5 and 9 distinct bioactive compounds in *C. collinus, P. glabrum* and *M. azedarch*, respectively (Fig 11-13). The Rf values of the bioactive principle of *C. collinus* varied from 0.11 – 0.91, while that of *P. glabrum* and *M. azedarch* varied in the ranges of 0.31 – 0.9 and 0.08 – 0.8, respectively. The different R_f values of the compounds separated in TLC and exposed in different light exposure were presented in the figure 7, 8, 9.

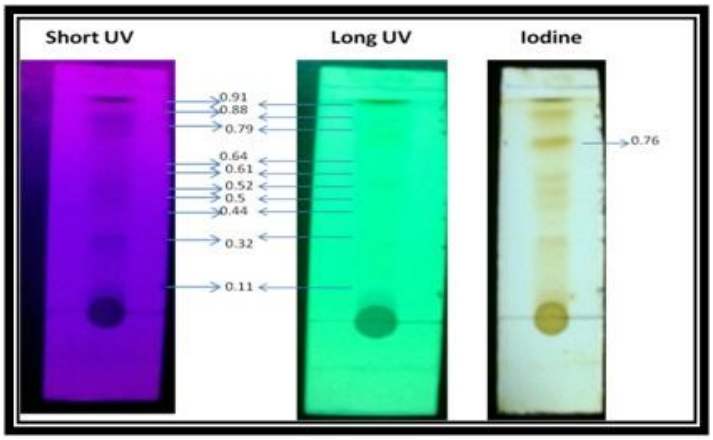

Figure 7: TLC profile of methanol extract of *C. collinus*

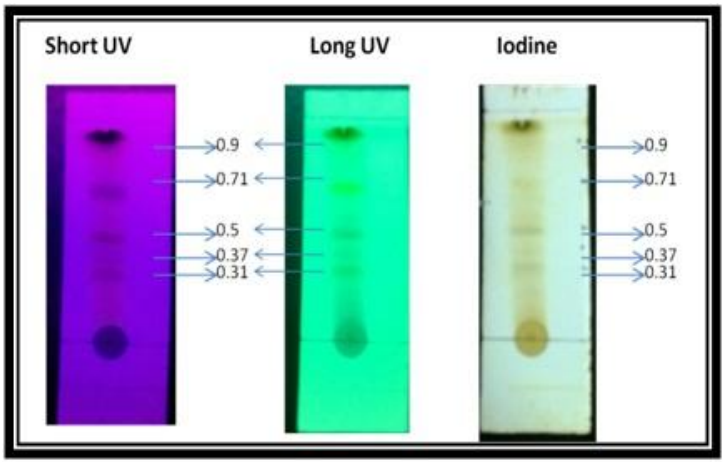

Figure 8: TLC profile of methanol extract of *P. glabrum*

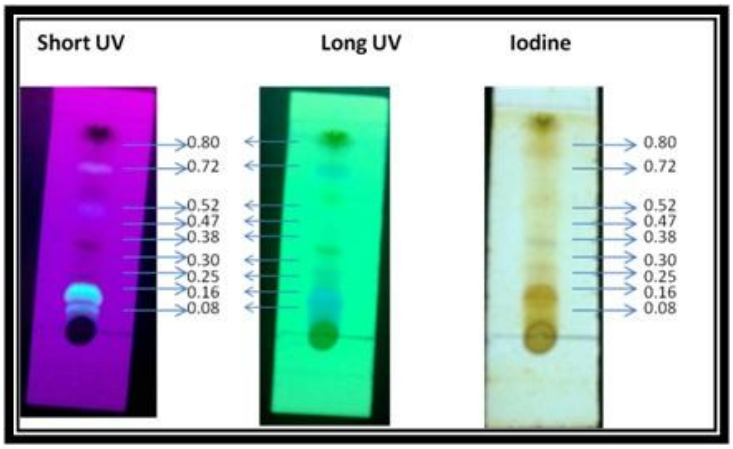

Figure 9: TLC profile of methanol extract of *M. azedarch*

4. Summary and Conclusion

Many naturally occurring compounds found in plants, herbs, and spices have been shown to possess antimicrobial functions and serve as a source of antimicrobial agents against pathogens (Deans and Ritchie 1987; Kumar *et al*., 2006). Bacterial infectious diseases represent an important cause of morbidity and mortality worldwide. Therefore, the development of new antimicrobial agents for the treatment of bacterial infections is of increasing interest. The main objective of the present study was to evaluate the ability of the plants extract to inhibit the growth of pathogenic bacteria and fungi to determine their phytochemical potential. Antimicrobial activity was recorded when the zone of inhibition is greater than 5 mm.

Almost all tested plant extracts showed antibacterial activity against *E.coli, S.aureus M. luteus* and *P.aurgenosa* which may reflect the antibacterial activity of plant active ingredients that inhibit bacterial growth. In our experiments, well diffusion method was used to assess the activity of plant extracts. The difference in antibacterial activity of a plant extract might be attributable to the age of the plant used, freshness of plant materials, physical factors (temperature, light water), time of harvesting of plant materials and drying method used before the extraction process. The antifungal activity of the plant extracts against *C. albicans* and *C. tropicali* tested, only *C. collinus* extract successful in inhibiting the fungal species with a maximum zone of inhibition of 10mm against *C. albicans* and *C. tropicalis* at a concentration of 200µg/ml.

However, the plants *P. glabrum* and *M. azedarch* not control the growth in the concentration range tested. This indicates the phytochemical compounds present in *P.glabrum* and *M. azedarch* do not have the antimicrobial property at significant level. The result of our study is in agreement with Seddik *et al*., (2010) and Mohamed *et al*., (2010) which demonstrated that *A. herba-alba* aqueous extracts had a antibacterial activity against *E.coli*. Plants antimicrobials have been found to be synergistic enhancers in that though they may not have any antimicrobial properties alone, but when they are taken concurrently with standard drugs they enhance the effect of that drug (Rakholiya and Chanda, 2012).

The result of phytochemical screening revealed the presence of flavonoids and tannins in all extracts. Flavonoids and tannins have been reported to possess antimicrobial activity, the antimicrobial activity of flavonoids is due to their ability to complex with extracellular and soluble protein and to complex with bacterial cell wall while that of tannins may be related to their ability to inactivate microbial adhesions, enzymes and cell envelop proteins (Cowan, 1999). In addition, the greater potency of methanol extracts was due to its ability to solubilize more antimicrobials from plants than ethanol and water (Eloff, 1998). Plants possess many phytochemical, which function as antioxidants and play an important role as health protecting agents. Such agents reduce the risk for chronic diseases like inflammatory cytokines and heal diseases. Few phytochemical such as phenolic acids flavonoids and polyphenols scavange free radicals such as peroxidase, hydroperoxidase, superoxidase, lipid peroxidase and inhibit major oxidative mechanisms. The antioxidant activity of various foods can be determined by DPPH method (Bhandari *et al.,* 2010). The medicinal plants contain phytochemical that have a healing effect by increasing the immune system response and detoxification and antioxidants activity of the body. Recent studies of tumor inhibiting compounds of plant origin have yielded an impressive array of novel structures of tumor inhibiting substances or compounds (Lavanya and Rama Devi 2008).

The plant material of *C. collinus, P. glabrum* and *M. azedarch* was successfully extracted by direct solvent extraction using methanol. The methanol extract of *C. collinus*s howed maximum activity against all the tested pathogens such as *S. aureus, M. luteus, E. oli*and *P. aeruginosa*, with a maximum ZOI of 15, 11, 12 and 18mm. The maximum inhibition was recorded against *P. aeruginosa* followed by *S. aureus, E. coli* and *M. luteus.* The antifungal potential of the extracts was not found to be significant, as only *C. collinus* showed inhibition of 11mm against both *C. albicans* and *C. tropicalis.* The antioxidant potential of the extracts was found potent since all the extracts showed remarkable radical scavenging activity. The phytochemical analysis revealed the presence of compounds like Flavonoids, phenols, tannins, terpenoids and reducing sugars. Thus the data obtained from the study suggests that the selected medicinal plants, *C. collinus, P glabrum* and *Melia azedarch* proved to be potent inhibitors of bacterial pathogens. However, further mechanistic studies are required to prove the exact mechanism behind the inhibition. Thus these plants could be considered as a significant source of natural antimicrobial agents.

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6. References

1. Abdel Rahman. S, Abd-Ellatif. S, Deraz. S and Khalil.A. Antibacterial activity of some wild medicinal plants collected from western Mediterranean coast, Egypt: Natural alternatives for infectious disease treatment. African Journal of Biotechnology, **2011**, Vol. 10(52), 10733-10743.
2. Abiramasundari.P, Priya .V, Jeyanthi.G.P, and GayathriDevi.S. Evaluation of the Antibacterial activity of Cocculushirsutus. Hygeia. Journal for Drugs and Medicines, **2011**, vol.3 (2), 26-31.
3. Beuchat LR, Golden DA. Antimicrobial occurring naturally in foods. Food Technol., **1989**, 43: 134-143.
4. Bhandari P, Kumar N, Singh B, Ahuja PS. "Online HPLC-DPPH method for antioxidant activity of *Picrorhziakurroa*Royle ex Benth. J. Exp. Biol., **2010**, 48(3): 323-328.

5. Chanda.S and Rakholiya.K (**2011**). Combination therapy: Synergism between natural plant extracts and antibiotics against infectious diseases. Science against microbial pathogens: communicating current research and technological advances A. Méndez-Vilas (Ed.).
6. Cowan ST, Steel S, (**1993**). Manual for the identification of Medical Bacteria. In Barrow GI, Feltham RKA (Ed.) Cambridge University Press. P. 32.
7. Deans. G and Ritchie G. Antibacterial properties of plant essential oils.International Journal of Food Microbiology, **1987**, 5, 165–180.
8. Elbashiti. T, Elmanama. A and Masad.A. The Antibacterial and Synergistic Effects of Some Palestinian Plant Extracts on Escherichia coli and Staphylococcus aureus. Functional Plant Science and Biotechnology, **2011,** Vol. 5 (1), 57-62.
9. Eloff, J.N., Which extractant should be used for the screening and isolation of antimicrobial components from plants? Journal of Ethnopharmacology, **1998**, 60: 1-8.
10. Gerald J. The herbal or general history of plants. Arco Pub. Co., New York, **1975**, pp.1-13.
11. Govindappa m, sadananda ts, channabasava r, jeevitha mk, pooja ks and vinay b. Raghavendra. Antimicrobial, antioxidant activity and phytochemical screening of tecoma stans (l.) Juss. Ex kunth Journal of phytology, **2011**, 3(3): 68-76.
12. Hegde Chaitra R., M. Madhuri1, Swaroop T. Nishitha1, Das Arijit1, Bhattacharya Sourav1 and K.C. Rohit. Evaluation of Antimicrobial Properties, Phytochemical Contents and Antioxidant Capacities of Leaf Extracts of Punica granatum L. ISCA Journal of Biological Sciences ISSN 2278-3202, **2012**, Vol. 1(2), 32-37.
13. Kumar. V, S. Neelam, Padh. H and Rajani.M. Search for antibacterial and antifungal agents from selected Indian medicinal plants. Journal of Ethnopharmacology, **2006**, 107: 182–188.
14. MadhuriS.andPandey G. Some anticancer medicinal plants of foreign origin. Current Science, **2009**, Vol. 96, No. 6, 25.
15. Mohamed.A, El-Sayed. M, Hegazy. M, .Helaly. S, Esmail1. A and Mohamed.N (**2010**).Chemical Constituents and Biological Activities of Artemisia herba-alba. Records of natural products page 1-25.
16. Meryem Seladji, Chahrazed Bekhechi, Fawzia Beddou, Hanane Dib, Nassima Bendimerad. Antioxidant activity and phytochemical screening of Nepeta nepetella aqueous and methanolic extracts from Algeria. Laboratory of Natural Products (LAPRONA), Journal of Applied Pharmaceutical Science **201**4, Vol. 4 (02), pp. 012-016, Available online at 10.7324/JAPS.2014.40203, ISSN 2231-3354.
17. Prabakaran M, N. Chandrakala and A. Panneerselvam, Antimicrobial activity of *Indigoferaglandulosa* (wild), Asian Journal of Plant Science and Research, **2011**, 1(2):18-25.
18. Seddik. K, Nadjet. I, Abderrahmane. B, Daoud. H and Lekhmici.A. Antioxidant and antibacterial activities of extracts from Artemisia herbaalbaAsso. leaves and some phenolic compounds. Journal of Medicinal Plants Research, **2010**, Vol. 4(13), 1273-280.
19. Shibasaki I. Food preservation with nontraditional antimicrobial agents. J. Food Saf., **1982**, 4:35-58.
20. Suman T, Chakkaravarthi K and R. Elangomathavan1. Phyto-chemical profiling of *Cleistanthus collinus* leaf extracts using GC-MS analysis, Research J. Pharm. and Tech. **2013**, 6(11), ISSN 0974-3618.
21. Tahiya Hilal Ali Alabri, Amira Hamood Salim Al Musalami, Mohammad Amzad Hossain , Afaf Mohammed Weli, Qasim Al-Riyami (**2013**). Comparative study of phytochemical screening, antioxidant and antimicrobial capacities of fresh and dry leaves crude plant extracts of *Datura metel* L. Journal of King Saud University – Science., 7:2.
22. Webb G., Herbs and spices with antioxidant and/or antimicrobial compounds. J. Amer. Botan. Council: **1997**, 39 pp.20.
23. Zoe Merculieff , Shubharani Ramnath , Seethalakshmi Madhukar Sankoli, Sivaram Venkataramegowda 2,4, Ganti S. 56,7Murthy , Ruben Michael Ceballos (**2014**). Phytochemical, antioxidant and antibacterial potential of Elaeagnus kologa (Schlecht.) 10.12980/APJTB.4.2014C1104.