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RESEARCH ARTICLE

STUDIES ON PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF *ACANTHACEAE SPECIES*

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ABSTRACT

Acanthaceae is a family of dicotyledonous flowering plants containing almost 250 genera and about 4,000 species. Acanthaceae is a family well known for its medicinal values due to the presence of valuable phytochemical compounds. The knowledge of the properties of the medicinal plants is growing as a result of which research and testing are being carried out throughout the world as they could be an alternative option for allopathic medicine. In the present study the analysis of Phytochemical compounds and antimicrobial activity of seven plant samples belonging to *Adathoda beddomie*, *Neelagiranthasis Sp*, *Justeaceae gendurusa*, *Neelagiranthasis hemitomie*, *Berberia priorities*, *Adathoda zylanica* and *Hemigraphis corolata* was analyzed where it was found that tannins were present in all the plant species which indicate that tannin plays a major role in the medicinal property of the plant species. The solvent extract showed antimicrobial activity against all the clinically isolated microorganisms. The maximum antagonistic effect was seen against *Pseudomonas* species.

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INTRODUCTION

The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed. Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies (Vaghasiya and Yogeshkumar, 2009). Herbal medicines are an essential and growing part of the international pharmacopeia. Knowledge of their medicinal properties is growing as a result of research and testing, which will make them an increasingly safe alternative or a preferred option to allopathic medicine. Today, there is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources. This revival of interest in plant-derived drugs is mainly due to the current widespread belief that "green medicine" is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects (Parekh and Chanda 2006). There is a growing interest in correlating phytochemical constituents of a plant with its pharmacological activity (Gupta 1994; Vaidya 1994). Scientists have even started correlating the botanical properties

of plants with their pharmacological activity (Rawat *et al.*, 1997). In future, more co-ordinated multidimensional research aimed at correlating botanical and phytochemical properties to specific pharmacological activities is expected (Dahanukar *et al.*, 2000).

Acanthaceae is a family of dicotyledonous flowering plants containing almost 250 genera and about 4,000 species. Most are tropical herbs, shrubs, or twining vines; some are epiphytes. Only a few species are distributed in temperate regions and among these, seven species were selected from the Karnataka region belonging to four genera namely *Adathoda*, *Justeaceae*, *Neelagiranthasis* and *Barleria*. Acanthaceae is a family well known for its medicinal values due to the presence of valuable Alkaloids, Phenols, Terpenoids, Tannins, Quinones, Cardiac glycosides, Saponins, Carbohydrates, flavonoids and Proteins. Various medicinal properties have been attributed to natural herbs. Medicinal plants constitute the main source of new pharmaceuticals and healthcare products. The history of plants being used for medicinal purpose is probably as old as the history of mankind. Extraction and characterization of several active phyto-compounds from these green factories have given birth to some high activity profile drugs. A growing body of evidence indicates that secondary plant metabolites play critical roles in human health and may be nutritionally important. Phytochemical screening of plants has revealed the presence of numerous chemicals including alkaloids, tannins, flavonoids, steroids, glycosides, saponins etc.

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Infectious diseases are the leading cause of death world-wide. Antibiotic resistance has become a global concern. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity. (Vaghasiya 2009)

MATERIALS AND METHODS

Sample collection

Plants belonging to acanthaceae family like *Adathoda beddomie*, *Neelagirianthisis Sp*, *Justeaceae gendurusa*, *Neelagirianthisis hemitomie*, *Berleria priorities*, *Adathoda zylanica* and *Hemigraphis corolata* were collected from FRLHT, Yelhanka, Bangalore in sterile bags and transported to the laboratory for further study on Phytochemical analysis and antimicrobial activity against several bacteria.

Solvent extraction

Leaves were air dried completely under shade. After complete air drying leaves were ground to fine powder and stored at room temperature. 1gm of each sample powder was added to 25ml of solvent and kept for 48hrs with slight shaking. Here, ethanol was used as a solvent. After 48hrs, extract were filtered by using whattmann no1 filter paper to get filtrate as extracts and were stored until further use.

PHYTOCHEMICAL ANALYSIS

Qualitative analysis of selected plants

Following standard protocols were used for qualitative analysis of samples to check for presence of Alkaloids, Carbohydrates, cardiac glycosides, Flavonoids, Phenols, Saponins, Tannins, Terpenoids, Quinones and proteins.

Test for flavonoids

To check the presence of flavonoids 2 ml of each extract was taken and added with a few drops of 20% NaOH this resulted in the formation of intense yellow colour. To this, a few drops 70% dilute HCl was added and this resulted in the disappearance of the yellow colour. The Formation and disappearance of yellow colour indicates the presence of flavonoids in the extract.

Test for Saponins

To test the presence of saponins 2 ml of each extract was added to 6 ml of distilled and shaken vigorously. The formation of bubbles or persistent foam indicates the presence of saponins.

Test for Tannins

10% of alcoholic ferric chloride was added to 2ml of the solvent extract. Formation of brownish blue or black colour indicates the presence of tannins.

Test for Phenols

To 2 ml of each extract, 2ml of 5% aqueous ferric chloride were added. Formation of blue colour indicates the presence of Phenols in the extract.

Test for Proteins

The presence of proteins was determined by adding 1ml of 40% NaOH and few drops of 1% copper sulphate to 2ml of the extract. The Formation of violet colour indicates the presence of peptide linkage molecule in the extracts.

Test for Cardiac Glycosides

1 ml of each extract was added to 0.5ml of glacial acetic acid and 3 drops of 1% aqueous ferric chloride solution. The Formation of brown ring at the interface indicates the presence of cardiac glycosides in sample

Test for Terpenoids

1ml of extract of each solvent was taken with 0.5 ml of chloroform followed by a few drops of concentrated sulphuric acid. Formation of reddish brown precipitate indicates the presence of terpenoids in the extract.

Test for Carbohydrates

1ml of Extract was added with a few drops of Molisch's reagent and then with 1ml of concentrated sulphuric acid at the side of the tubes. The mixture was then allowed to stand for 2 to 3 minutes. Formation of red or dull violet colour indicates the presence of carbohydrates in the extract.

Test for Quinones

To check the presence of quinones 1ml of extract added to concentrated Hydrochloric Acid. The Formation of yellow precipitate or Coloration indicates the presence of Quinones in the Extract. Otherwise indicates absence of Quinones in the given extract

QANTITATIVE ASSAY

Depending on above qualitative results the quantitative assay is carried out for Alkaloids, Tannins, phenols, Proteins and Carbohydrates.

Total Tannins Content Determination

The tannins were determined by slightly modified Folin and Ciocalteu method. Briefly, 0.5 ml of sample extract is added with 3.75 ml of distilled water and adds 0.25 ml of Folin Phenol reagent, 0.5 ml of 35% sodium carbonate solution. The absorbance was measured at 725 nm. Tannic acid dilutions (0 to 0.5mg/ml) were used as standard solutions. The results of tannins are expressed in terms of tannic acid in mg/ml of extract.

Total phenol content Determination

The phenols were determined by slightly modified Folin and Ciocalteu method. Briefly, to the 200µl of the sample extract +800 µl of F. c reagent mixture add 2ml of 7.5% sodium carbonate then dilute the total content to 7 volumes with distilled water finally keep the tubes for 2hrs incubation in dark. The absorbance was measured at 765 nm. Gallic acid dilutions (0 to 0.5mg/ml) were used as standard solutions. The results of phenols are expressed in terms of gallic acid in mg/ml of extract.

Total protein content determination

The total proteins content was determined by using Bradford's method. Briefly, to the 100µl of the sample extract add 3ml of Bradford's reagent and incubate in dark for 5mints. The absorbance was measured at 595nm. Bovine serum albumin dilutions (0.1mg/ml to 0.5mg/ml) are used as standard solutions.

Total Alkaloid content determination

40 ml of 10% acetic acid in ethanol was added to 1g of powdered sample, covered and allowed to stand for 4 hours. The filtrate was then concentrated on a water bath to 1/4th of its original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle; collected precipitate was washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed.

Total Carbohydrate determination

For estimating the polysaccharide content, add 1ml of 5% phenol to the 1ml of sample solution, and then add 5ml of concentrated H₂SO₄ and measure the absorbance after 10 minutes at 488nm against blank. Then compare it with standard solution of glucose. To prepare Blank, 1ml of distilled water added to 1ml of 5% phenol followed by 5ml of Concentrated H₂SO₄.

ANTIMICROBIAL ASSAY

An antimicrobial or antibiotic agent is a substance/chemical that kills microorganisms or inhibits their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibacterial agents are used against bacteria and anti-fungal are used against fungi. They can also be classed according to their function. Antimicrobials that kill microbes are called *microbiocidal*, those that merely inhibit their growth are called *microbiostatic*. The antimicrobial assay was performed by agar disc diffusion method (Bauer *et al.*, 1966). The molten MuellerHinton Agar was prepared and poured into the sterile Petri plates.

Target microorganisms

Antimicrobial activity is performed against three different organisms namely *E.coli*, *Staphylococcus aureus* and *Pseudomonas spp* which were isolated from clinical samples collected from pathology labs in Bangalore.

Agar well diffusion method

The antimicrobial activity of the Phytochemical Extracts was determined by using the Agar Well Diffusion technique. Nutrient agar plates were each seeded with 0.5 ml of an overnight culture of each bacterial strain. The 24 hrs broth culture (0.1ml) of each bacterium was inoculated onto Muller Hinton agar plates by spread plate technique and well made by sterile cork borer and 80 µl (0.05 ml) solution of concentrated Plants extracts was added in to each well. (Mukesh Chandra Sharma and Smita Sharma, 2010, Borah *et al.*, 2013)

RESULTS

The results of total phenolic content and Tannins content, protein content, alkaloid content and carbohydrate content of ethanol extracts of 7 plants are presented in the following Tables 1-2.

Alkaloids

The results of total alkaloid content of all the ethanol extracts are shown in the following table. Among all the seven plant samples *Adathoda beddomie* and *Barleriapronities* shows more alkaloid content (Figure 1).

Tannins

The results of total content of tannins of samples of ethanol extracts are shown in the following table. Among all the plant samples *Barleriapronities* and *Neelagirianthisis hemietomi* has higher concentration of tannins compared with the other plant samples.

Table-1. Preliminary qualitative phytochemical analysis

Samples	Alkaloids	Carbohydrates	Cardiac glycosides	Flavonoids	Phenols
<i>A. Beddomieae</i>	+	+	-	-	-
<i>Nilgiranthus sp.</i>	+	+	-	-	-
<i>J. gendurussa</i>	+	+	+	-	-
<i>N. Hematoma.</i>	-	+	+	-	-
<i>Barleriapronities</i>	-	+	+	-	-
<i>Adathoda zylanica</i>	+	+	-	-	+
<i>H. Corolata</i>	+	+	+	-	+

Table-2. Preliminary qualitative phytochemical analysis

Samples	Saponins	Tannins	Terpenoids	Quinones	Proteins
<i>A. Beddommeae</i>	-	+	-	-	+
<i>Nilgiranthus sp.</i>	-	+	-	-	+
<i>J. gendurussa</i>	-	+	-	-	+
<i>N. Hematoma.</i>	-	+	-	-	+
<i>Barleriapronities</i>	-	+	+	-	+
<i>Adathoda zylanica</i>	-	+	-	-	+
<i>H. Corolata</i>	-	+	+	+	+

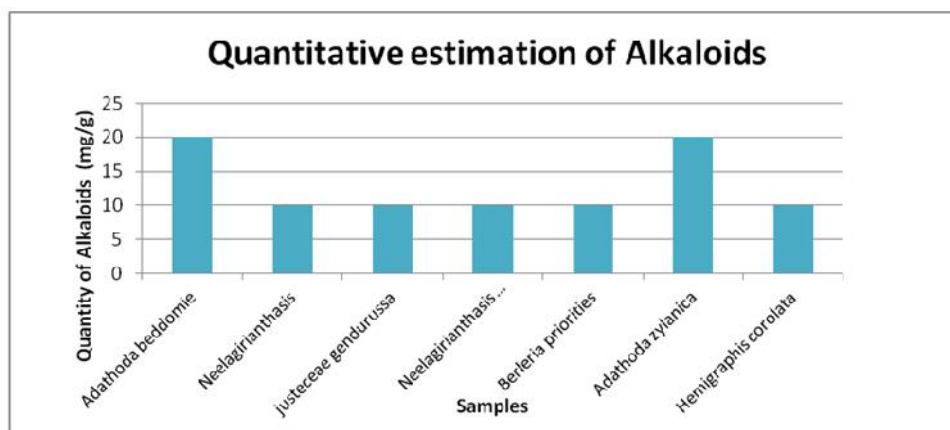


Figure-1. Quantitative estimation of Alkaloids

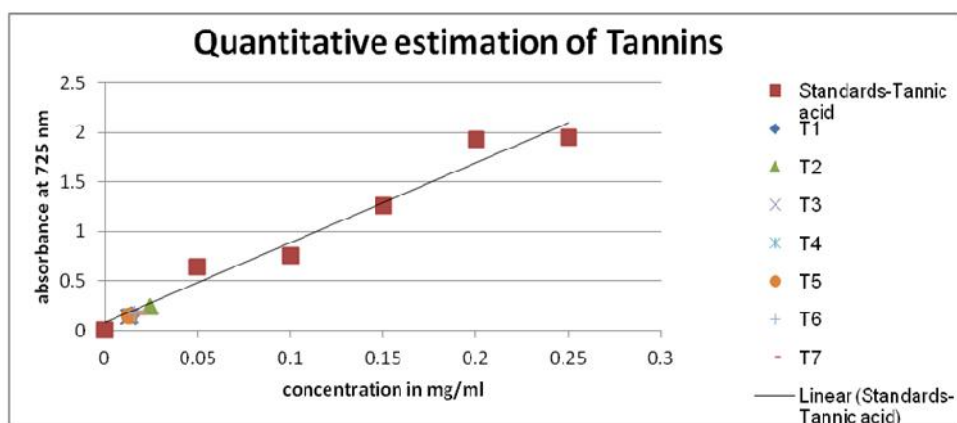


Figure-2. Standard Graph for Tannins

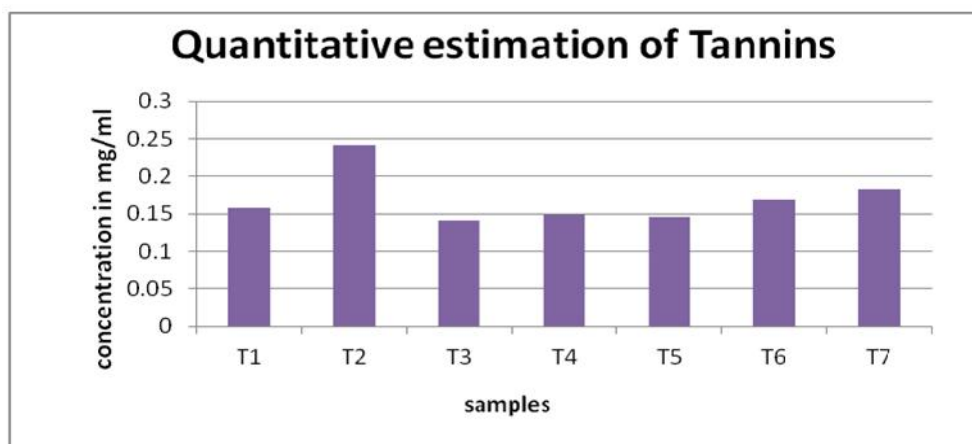


Figure-3. Graph showing quantitative estimation of Tannins

Phenols

The results total phenolic content of all samples of ethanol extracts are shown in the table below, among the seven samples only four samples shows phenolic activity (Figure 5).

Proteins

The results of total protein content of all the samples of ethanol extracts are shown in the following table, among all the seven samples T3 and T4 shows higher concentration of protein content (Figure 7).

Carbohydrates

The results of the total carbohydrate content of all the seven ethanol extracts are shown in the following Figure 9.

In the above all data T1, T2, T3, T4, T5, T6 and T7 are *Adathoda beddomie*, *Neelagirianthisis*, *Justecea gendurusa*, *N.hematome*, *Barleriapronities*, *Adathoda zylanica* and *Hemigraphis corolata* respectively.

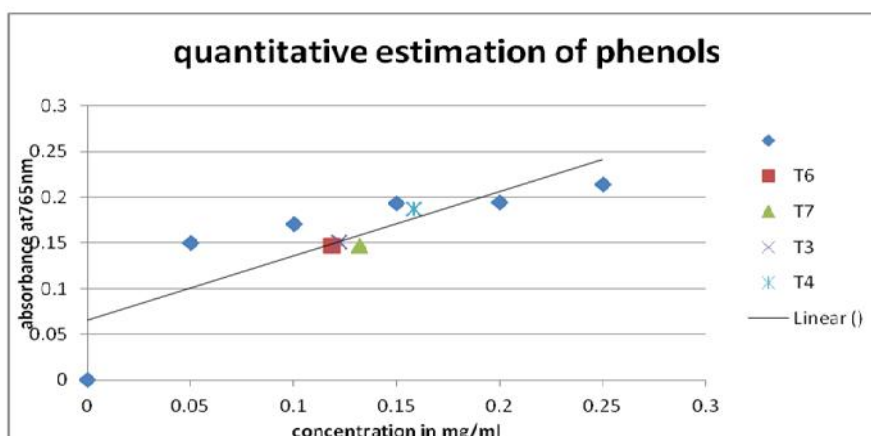


Figure-4. Standard Graph for phenols

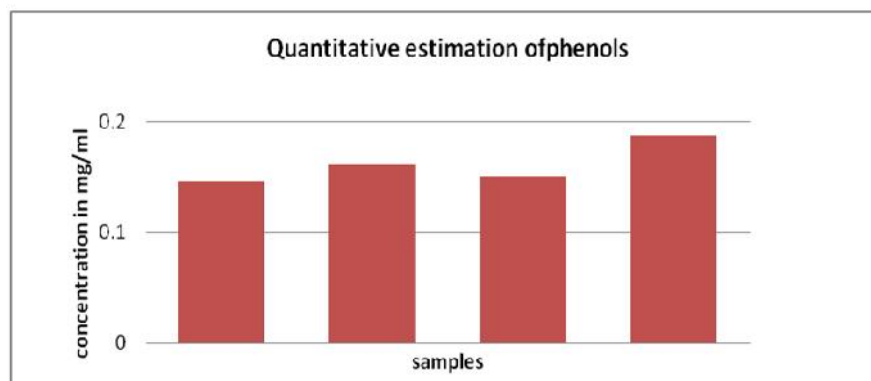


Figure-5. Graph showing quantitative estimation of Phenols

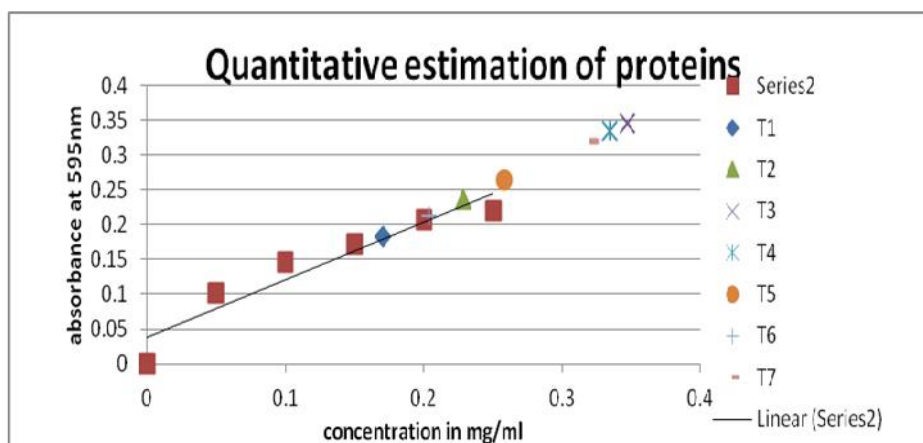


Figure-6. Standard Graph for proteins

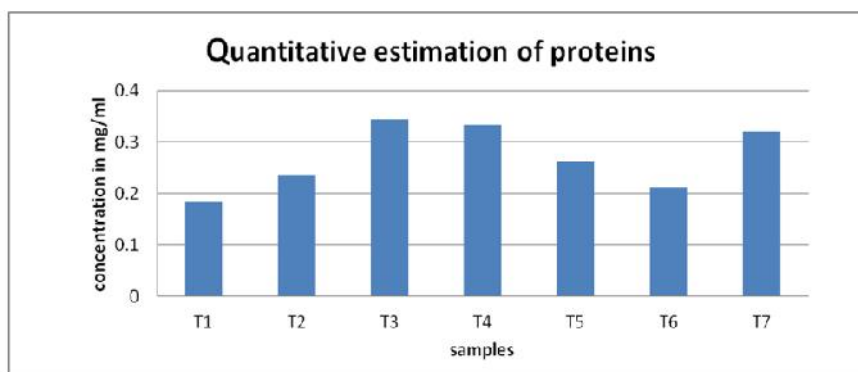


Figure-7. Graph showing quantitative estimation of Phenols

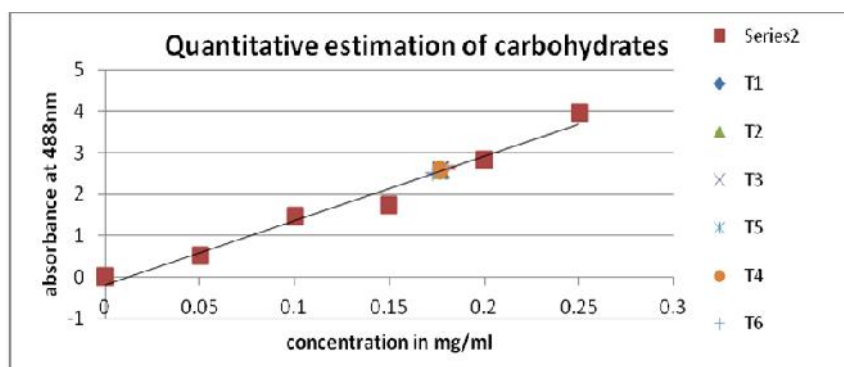


Figure-8. Standard Graph for carbohydrates

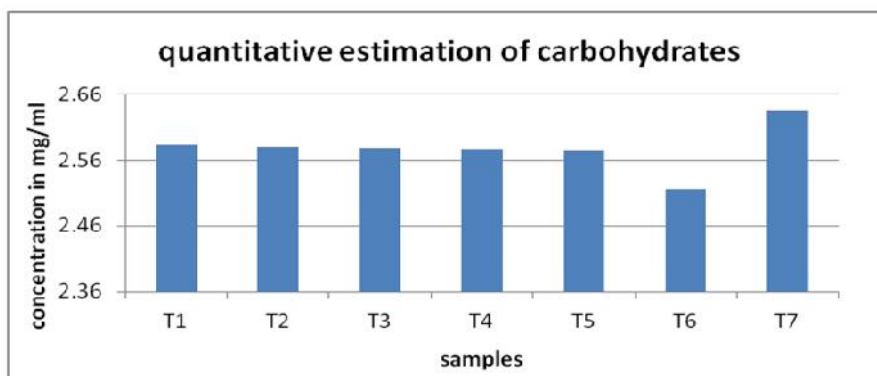


Figure-9. Graph showing quantitative estimation of carbohydrates

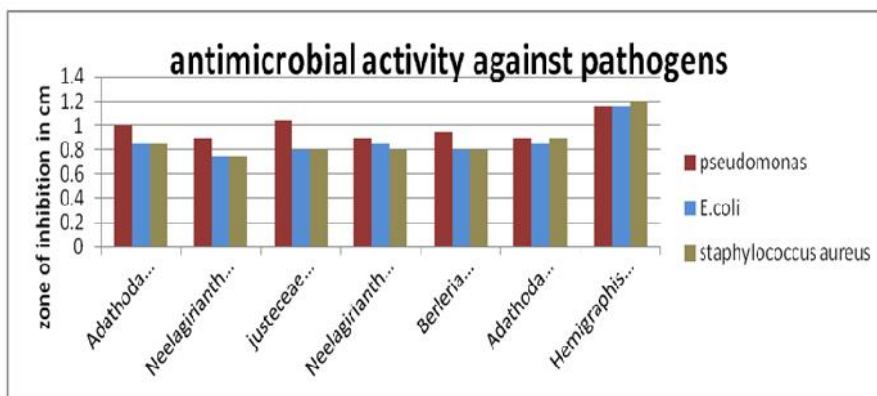


Figure-10. Antimicrobial activity against pathogens

Antimicrobial assay

Disc diffusion method is the most widely used procedure for testing antimicrobial susceptibility (Sambath Kumar *et al.*, 2006). The disc diffusion procedure (Kirby-Bauer method) has been accepted by the Food and Drug Administration (FDA) and as a standard by the National Committee for Clinical Laboratory Standards (Barry and Thornsberry 1985; NCCLS 2003). In the present study, ethanol extracts of 7 plants were studied against 3 standard strains of microorganisms, namely *E.coli*, *Pseudomonas*, *Staphylococcus aureus*. The ethanol was used as control. Following Figure 10 shows the antimicrobial activity of the seven samples of ethanol extracts against three most commonly used pathogens *Pseudomonas*, *E.coli* and *Staphylococcus aureus*.

Among all the seven ethanol extracts *Hemigraphis corolata* shows higher zone of inhibition against the three pathogens what I previously mentioned.

DISCUSSION

The phytochemical studies provide health application at an affordable cost. The study such as ethno medicine keenly represents one of the best avenues in searching new economic plants for medicine. Phytochemical studies and antimicrobial properties of various extracts from many plants have recently been of great interest in both research and the food industry, because their possible use as natural additives emerged from a growing tendency to replace synthetic antioxidants and antimicrobials with natural ones (Deba *et al.*, 2008). A comparative study of phytochemical compounds and antimicrobial activity of leaf extracts of seven herbal plants namely *Adathoda beddomei*, *Adathoda zylanica*, *Barleria prionities*, *Justice gendarussa*, *Nilgianthus sp.*, *Neelagiranthasis hemitomie* and *Hemigraphis cholorata* was aim of the study. The leaf extracts were prepared using ethanol as a solvent for phytochemical extraction. The qualitative analysis of phytochemicals encompasses alkaloids, flavonoids, terpenoids, tannins, saponins, quinones, phenols, protein, cardiac glycosides and carbohydrates. The presence of the various phytochemicals illustrates the importance of the selected seven species which can be used to cure various ailments.

Alkaloids were absent in *Nigiranthushematomie* and *Barleria prionities* as there was no formation of precipitate after performing the tests. Formation of red-violet ring in all samples in carbohydrates indicated presence of Carbohydrates in all the samples. Similarly, tannins and proteins were also present in all samples. Brown ring formation only in four samples namely *Neelagiranthasis hematomie*, *Barleria prionities*, *Hemigraphis cholorata* and *Justiceae gendarussa* indicated the presence of cardiac glycosides. Formation of black color indicated the presence of phenols in *Hemigraphis cholorata* and *Adathoda zylanica* samples. Whereas only two samples *Barleria prionities* and *Hemigraphis cholorata* showed presence of terpenoids and *Hemigraphis cholorata* showed positive result for Quinones. Flavonoids and Saponins were found to be absent in all the seven samples.

Among all seven samples *Hemigraphis corolata* and *Adathoda beddomei* showed higher concentration of carbohydrates. Carbohydrates were present in highest quantity i. e. 0.18mg/ml in *Hemigraphis colorata* compared to others whereas *Adathoda zylanica* showed least quantity of carbohydrates i. e. 0.172mg/ml. In total protein estimation, extract of *Justice gendarussa* showed highest protein content of 0.347mg/ml and *Adathoda beddomei* was found to have the least protein content of 0.17mg/ml. In total Tannin estimation, extract of *Neelagiranthasis sp.* showed highest quantity as 0.0243mg/ml and compared to others *Justice gendarussa* showed least quantity of tannins as 0.0125mg/ml. In total phenol estimation, extract of *Neelagiranthasis hemitomie* showed highest quantity as 0.148mg/ml where as compared to others *Adathoda zylanica* showed least quantity as 0.118mg/ml.

Antimicrobial activity was performed using the seven sample extracts against three common pathogens such as *E. coli*, *S. aureus* and *Pseudomonas*. Overall antimicrobial activity of these plants extracts seem to be less as compared to what stated in earlier literature. All the samples showed significant antimicrobial activity especially against *Pseudomonas sp.* but least against *S. aureus* and *E. coli*. Against *Pseudomonas* strains, *Hemigraphis corolata* showed highest antimicrobial activity with zone of inhibition of 1.15cm as compared to other samples whereas *Adathoda zylanica* showed least activity with zone of inhibition of 0.9cm.

Conclusion

The results of the preliminary qualitative phytochemical study of the seven crude samples of ethanol extracts shows that the presence of carbohydrates and proteins in all the seven ethanol extracts, except *Neelagiranthasis hemitomie* and *Barleria prionities*. All the other samples have alkaloids, except *Adathoda beddomei*, *Neelagiranthasis sp.* and *Adathoda zylanica*. Only *Neelagiranthasis hematomie*, *Barleria prionities*, *Hemigraphis cholorata* and *Justiceae gendarussa* showed presence of cardiac glycosides. As seen in results, most of the sample extracts were negative to phenols whereas only *Adathoda zylanica* and *Hemigraphis corolata* showed presence of phenols. Ethanol successfully extracted tannins from all the samples. Among the seven extracts, only *Barleria prionities* and *Hemigraphis corolata* have the terpenoids whereas ethanol extract of only *Hemigraphis corolata* showed presence of Quinones. Hence we conclude that ethanol is a suitable solvent to extract Tannins, carbohydrates and Proteins but its capacity to extract other phytochemicals varies in different selected plant species.

The results of the invitro quantitative phytochemical study of the seven crude samples of ethanol extracts for estimation of Carbohydrates, Proteins, Tannins and other present phytochemicals showed varying results. Ethanol extracts of all the selected samples were found to be rich source of Carbohydrates, Tannins and Proteins. The results of Antimicrobial activity of the seven crude samples of ethanol extracts shows *Hemigraphis corolata* has the higher antimicrobial activity followed by *Adathoda zylanica*, *Justiceae gendarussa*, *Adathoda beddomei*, *Neelagiranthasis hematomie*, *Barleria prionities* and *Neelagiranthasis sp.* against

the three common pathogens namely *Pseudomonas*, *E.coli* and *Staphylococcus aureus*. From the results antimicrobial assay, we can conclude that ethanol may not be a suitable solvent for extracting antimicrobial compounds or in other case, selected plant species may not be highly active against selected pathogens.

The phytochemical screening carried out in this study shows that these plants are rich in secondary metabolites which could be explored as potential drug leads and phytomedicine. The plants studied here can be seen as a potential source of new useful drugs because of presence of certain phytochemicals such as cardiac glycosides and Tannins. The phytochemical characterization of the extracts, the identification of responsible bioactive compounds and quality standards are necessary for future study.

REFERENCES

- Ahmad I, Aqil F, Owais M. 2006. Modern Phytomedicine. WILEY-VCH Verlag GmbH and Co. KGaA, Weinheim.
- Ajaiyeobu EO. 2002. Phytochemical and antibacterial activity of *Parkia biglobosa* and *Parkia bicolor* leaf extracts. *African Journal of Biomedical*
- Akerele O. 1993. Summary of WHO guidelines for the assessment of herbal medicines. *Herbal Gram* 28: 13-19
- Balandrin MF, Klinghorn AD, Farnsworth NR 1993. In: Human Medicinal Agents from Plants. Kinghorn AD, Balandrin MF (Eds.), American Chemical Society, Washington, DC pp. 2-12.
- Baravalia Y, Kaneria M, Vaghasiya Y, Parekh J, Chanda S 2009. Evaluation of antioxidant and antibacterial activity of *Diospyrosebenum* Roxb. Leaf (Ebenaceae). *Turkish Journal of Biology (In Press)*
- Bauer et al. 1966. NCCLS 2003, Parekh and Chanda 2007
- Bhattacharjee SK. 1998. Handbook of Medicinal Plants. Pointer Pub, Jaipur, India pp.
- Borah M., Swarnamoni Das, Ahmed S. 2013. Antibacterial Activity of the Ethanolic Extract of Leaves of Citrus Maxima (Burm.) Merr on Escherichia Coli And Pseudomonas Aeruginosa. *Asian J Pharm Clin Res*, Vol 6, Suppl 4, 136-139
- Brown JE, Rice-Evans CA. 1998. Luteolin rich artichoke extract protects lowdensity lipoprotein from oxidation in vitro. *Free Radical Research* 29: 247-255.
- Campos AR, Lima Jr, RC, Uchoa DE, Silveira ER, Santos FA, Rao VS. 2006. Pro-erectile effects of an alkaloidal rich fraction from *Aspidosperma* root bark in mice. *Journal of Ethnopharmacology* 104: 240-244.
- Cavalcanti BC, Costa-Lotufo LV, Moraes MO, Burbano RR, Silveira ER, Cunha KM, Rao VS, Moura DJ, Rosa RM, Henriques JA, Pessoa C 2006.
- Charaka Samhita 1949. Shree Gulab Kunverba Ayurvedic Society, Jamnagar, India
- Dahanukar SA, Kulkarni RA, Rege NN 2000. Pharmacology of medicinal plants and natural products. *Indian Journal of Pharmacology* 32: S81-S118.
- Deba F, Xuan TD, Yasuda M, Tawatu S. 2008. Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from *Bidens pilosa*. *Linn Var Radiata Food contrl.* 19: 346-352.
- Dev S. 1997. Ethnotherapeutic and modern drug development: The potential of Ayurveda. *Current Science* 73: 909-928.
- Dev S. 1999. Ancient modern concordance in Ayurvedic plants: some examples. *Environmental Health Perspectives* 107: 783-789.
- Evaluation of antioxidant and antimicrobial properties of *Soymida febrifuga* leaf extracts. *Phytotherapy Research* 22: 943-947.
- Garde GK. 1954. Sartha Vagbhatta, Ashtangahridaya. Aryabhushana Mudranalaya Pune, India.
- Genotoxicity evaluation of kaurenoic acid, a bioactive diterpenoid present in Copaiba oil. *Food and Chemical Toxicology* 44: 388-392.
- Gupta SS 1994. Prospects and perspectives of natural plant products in medicine. *Indian Journal of Pharmacology* 26: 1-12.
- Mukesh Chandra Sharma and Smita Sharma 2010. Phytochemical Screening and In Vitro Antimicrobial Activity of Combined Citrus paradisi and *Ficus carica* Linn Aqueous Extracts, *International Journal of Microbiological Research* 1 (3): 162-165
- Reddy BS, Reddy BP, Raghavulu SV, Ramakrishna S, Venkateswarlu Y, Diwan PV 2008. *Research* 5: 125-129.
- Somolenski SJ, Silinis H, Farnsworth NR 1972. Alkaloid screening. I. *Lloydia* 35: 1-34.

