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Phytochemical Analysis and their Antibacterial Efficacy of some Medicinal Plants of the Local Areas of Tumakuru

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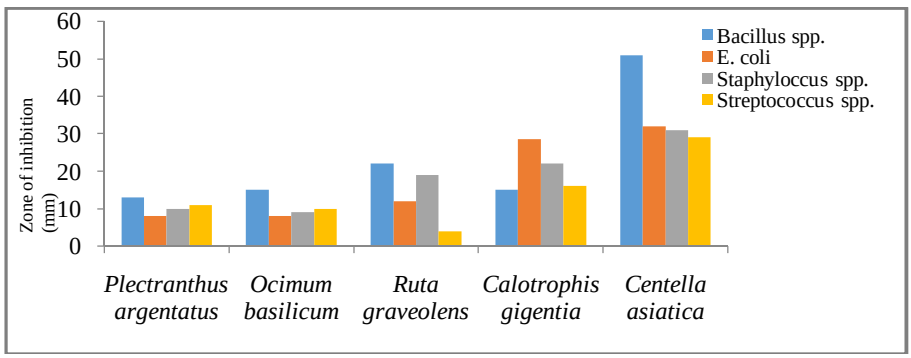
ABSTRACT

Microorganisms are becoming resistant to drugs used to kill them, hence the need for alternative drugs to treat them. Phytochemicals have been associated with reduction of drug resistant forms of bacteria. Pharmacological studies have reported appealing results showing the importance of using plant extract to treat diseases. The present study explores the photochemical and antibacterial efficacy of leaf extracts of Plectranthus argentatus, Ocimum basilicum, Rutagraveolens, Calotrophis gigentia, and Centella asiatica. The phytochemical analysis revealed that major phytoconstituents of Plectranthus argentatus are alkaloids, terpoinoids, phenols, tannins, flavonoids and steroids. Ocimum basilicum L. revealed the presences of glycosides, tannins, Saponins terpenoids and phenols. Rutagraveolens L. has shown the presence of steroids, flavonoids, phenols, tannins, terpenoids and saponins. Phytochemical analysis of Calotrophis gigentia showed the presence of alkaloids, phenols, saponin, and steroids. Similarly, Centella asiatica revealed the presence of flavanoids, tannins, terpenoid, saponin and steroids. The antibacterial activity of the plant extracts was screened by agar well diffusion method against the bacterial strains such as Bacillus, E. coli, Staphylococcus and Streptococcus respectively. Hence, further work has to be carried out to isolate and identify the active constituents of the plants responsible for antibacterial activity.

Graphical Abstract



Antibacterial activity of plant extracts against some human pathogens

Keywords: Medicinal plants, Photochemical, Antibacterial efficacy, Phytoconstituents, Zone of inhibition.

INTRODUCTION

In India, 95% of the traditional system prescriptions of Unani, Ayurveda, Homeopathy and Siddha are plant based chemicals [**1**]. The plant based chemical compounds are classified into two classes; primary and secondary metabolites based on their chemical, biosynthetic origin and functional groups. Primary metabolites are involved in growth and development and secondary metabolites are involved in defense mechanism against harmful pests and infectious agents. The later class exhibit medicinal properties. Plant derived chemicals such as terpenoids, phenolics, alkaloids, flavanoids, glycosides and minor chemicals are having better compatibility with human body. It is estimated that 30% of the worldwide sales of drugs are based on plant products [**2**]. Many drug resistant microbes are emerging from time to time and causing the need to such for new antibiotics to kill and inhibit their growth. Phytochemicals have been associated with reduction of drug resistant forms of bacteria.Tumkur District, Karnataka, reports 918 taxa including 26 infra-specific taxa falling under 504 genera and 139 families, encompassing 13 fern and 1 gymnosperm families [**3-5**]. The "Dhanvantrivana" of Tumkur University campus also has number of medicinal plants. In keeping this view in mind the present investigation is designed to explore preliminary phytochemical analysis andantibacterial efficacyof aqueous extract of leaves of *Plectranthus argentatus, Ocimum basilicum, Rutagraveolens, Calotrophisgigentia,* and *Centellaasiatica*of local regions of Tumkur district, Karnataka, India.

MATERIALS AND METHODS

Collection and preparation of plant material: Leaves of the selected plant material such as *Plectranthus argentatus, Ocimum basilicum, Rutagra veolens, Calotrophi sgigentia,* and *Centella asiatica* were collected from "Dhanavantrivana" botanical garden of University campus. The leaves were air dried for 7-10 days in ashade prevent the loss of active phytoconstituents and groundinto fine powder using a mechanical grinder.

Aqueous extraction: The powdered plant material (25 g) were soaked in 100 mL of distilled water and boiled at 50-60°C for 30 min on water bath. The extract was filtered through Whatman No.1 filter paper and centrifuged the filtrate at 2500 rpm for 15 min. Resulting extract was stored in sterile bottles at 4-8°C for further analysis [**6**]. One hundred grams of the thoroughly washed and air dried healthy.

Qualitative phytochemical analysis: The extracts phytochemical analysis for identification of bioactive chemical constituents was done using standard procedures

Tannins: About 0.5 g of the sample was put in a test tube and 20 mL of distilled water was added and heated to boiling. The mixture was then filtered and 0.1 % of $FeCl_3$ was added to the filtrate and observations made. A brownish green color or a blue black coloration indicate the presence of tannins.

Saponins: The crude solvent extract was mixed with 5 mL of water and vigorously shaken. The formation of stable foam indicates the presence of saponins.

Flavonoids: About 1g of the plant extract was mixed with a few fragments of magnesium ribbon (0.5 g) and a few drops of concentrated hydrochloric acid were added. A pink or magenta red color development after 3 minutes indicate the presence of flavonoids.

Terpenoids: The solvent extracts of the plant material was taken in a clean test tube 2 mL of chloroform was added and vigorously shaken, then evaporated to dryness. To this, 2 mL of

concentrated sulphuric acid was added and heated for about 2 minutes. A greyish color indicates the presence of tarpenoids.

Glycosides

Salkowski test: 2 mL of extract was treated with 2 ml of acetic anhydride. Few drops of concentrated sulfuric acid was then added to this solution and observed the formation of blue, green rings that indicates the presence of terpenoids.

Alkaloids: 2 mL of extract was treated with 2 drops of Mayer's reagent. Presence of white creamy precipitate indicates the positive test.

Steroids

Libermann-Burchard reaction: About 2 g of the solvent extract was put in a test tube and 10 mL of chloroform added and filtered. Then 2 mL of the filtrate was mixed with 2 ml of a mixture of acetic acid and concentrated sulphuric acid. Bluish green ring indicate the presence of steroids.

Phenols: The plants solvent extract was put in a test tube and treated with a few drops of 2% of $FeCl_3$; blue green or black coloration indicate the presence of phenols.

Coumarins: 2 mL of extract was treated with 3 mL of 10%NaOH, observed the formation of yellow color indicating the presence of coumarins**.**

Anthocyanins: 2 mL of extract was treated with 2 mL of 2N hydrochloric acid and ammonia was added to it, observed the appearance of pink-red color turning blue-violet. This indicates the presence of anthocyanins.

Quinones: 1 mL of extract was added to the 2 mL of dilute NaOH. Formation of blue green or red coloration confirms the presence of Quinones.

Preparation of test microorganisms: The pathogenic microbial strains namely *Staphylococcus spp., E. coli, Bacillus spp. and Streptococcus spp.* were subcultured on nutrient agar and were used for the antibacterial activity.

Well diffusion method: An overnight culture of test bacteria was grown into 20 mL of nutrient broth. The sterile Nutrient agar medium was poured into the petri plates and allowed to set. Thereafter, all the inoculum samples was swabbed over the surface of the nutrient agar medium using sterile cotton swab. Using a sterile cork borer of 5 mm diameter, five wells were made in solidified sterile nutrient agar medium (one in the centre and four wells at the corner). The agar plugs were removed with a flamed and cooled wire loop. Then 50µL aqueous extract of leaves were poured in the wells made in inoculated plates. The treatment includes 50 µL of distilled water served as control. All the plates were incubated for 24 h at 37°C and zone of inhibition if any around the well were measured in millimeter (mm). The same procedure was followed for standard antibiotics chloramphenicol (25 mg) and Tetracycline (25 mg) to compare the efficacy of plant extract against test organisms.

RESULTS AND DISCUSSION

Anti-microbial properties of plant extracts have been treated as new classes of antibiotics [7]. Five plants *Plectranthus argentatus, Ocimum basilicum, Rutagra veolens, Calotrophis gigentia,* and *Centella asiatica* were screened for their phytochemical constituents and the results were presented in table 1. The antimicrobial activity of plants extract was investigated against various pathogenic organisms such as *Bacillus spp., Escherichia coli, Staphylococcus spp. and Streptococcus spp.* using well diffusion method. The diameter of inhibition zones (mm) around each well were shown in the figure 1. The important thing is that all plant samples contain one common and abundant secondary

metabolite, flavonoid. From the literature survey it was found that flavonoids have wide range of biological properties such as anti-inflammatory, antibacterial, antiviral, anti-allergic, cytotoxic antitumor properties. Alkaloids and phenolic compounds along with hypoglycemic, antidiabetic properties also exhibit antiinflammatory, antimicrobial and antioxidant effects [**8-10**].

Table 1. Preliminary phytochemical analysis of screened medicinal plant species

S.No.	Plant extracts	*Plectranthus argentatus*	*Ocimum basilicum*	*Rutagra veolens*	*Calotrophis gigentia*	*Centella asiatica*
1	Phenols	+	+	+	+	-
2	Alkaloids	+	-	-	+	-
3	Tannins	+	+	+	-	+
4	Terpenoids	+	+	+	-	+
5	Flavonoids	+	-	+	-	+
6	Steroids	+	-	+	+	+
7	Glycosides	+	-	-	-	-
8	Saponins	+	+	+	+	+
9	Coumarins	+	-	+	+	+
10	Anthocyanins	-	-	-	-	-
11	Quinones	-	+	+	+	-

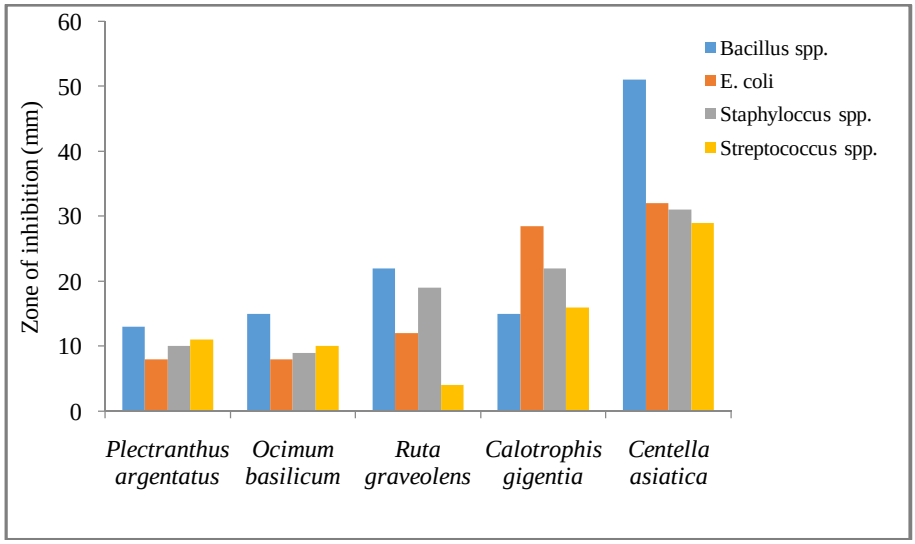


Figure 1. Antibacterial activity of plant extracts against some human pathogens.

APPLICATION

Flavonoids are associated with a broad spectrum of health-promoting effects because of their antioxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme functions [**12-14**]. Cardiac glycosides such as digitoxin, digoxin, and convallotoxin support heart strength and rates of contraction when failing. These compounds also have a diuretic effect that stimulates urine production and aids in removal of fluid from tissues and the circulatory system.

CONCLUSION

The medicinal properties are due to the high steroids, flavonoids, phenols, tannins, terpenoids and saponins. Hence, further work has to be carried out to isolate and identify the active constituents of the plants responsible for antibacterial activity.

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REFERENCES

[1]. N. Savithramma, M. Linga Rao, D. Suhrulatha, Screening of Medicinal Plants for Secondary Metabolites, *Middle-East J. Sci. Res*., **2011**, 8(3), 579-584.
[2]. G. V. Satyavati, A. K. Gupta, N. Tandon, Medicinal plants of India, Indian Council of Medical Research, New Delhi, India, **1987**.
[3]. B. Patwardhan, A.D.B. Vaidhya, M. Chorghade, Ayurveda and Natural products drug discovery, *Curr Sci.*, **2004**, 86: 789-799.
[4]. Veeralinga Bhaskar, Khushalappa, K. S. Jagadishchandr, Flora of Tumkur District Karnataka, Centre for Plant Taxonomic Studies, Bangalore , **2014**, 2, 369.
[5]. P. Shah, H. A. Modi, M. D. Shukla, S. K. Lahiri, Preliminary Phytochemical Analysis and Antibacterial Activity of Ganoderma lucidum collected from Dang District of Gujarat, *India. Int. J. Curr. Microbiol. App.Sci.*, **2014**, 3(3), 246-255.
[6]. A. Soni, S. Sosa, Phytochemical Analysis and Free Radical Scavenging Potential ofHerbal and Medicinal Plant Extracts, *J. Pharma. Phytochem.*, **2013**, 2 (4), 22-29.
[7]. N. A. Ali, W. D Julich, C. Kusnick, U. Lindequist, Screening of Yemeni medicinal plants forantibacterial and cytotoxic activities, *J. Ethnopharmacol*., **2001**, 74, 173-17.
[8]. S. S. Dakshayani, M. B. Marulasiddeshwara, M. N. Sharath Kumar, Golla Ramesh P.Raghavendra Kumar, S. Devaraja Rashmi Hosamani, Antimicrobial, anticoagulant and antiplatelet activities of green synthesized silver nanoparticles using Selaginella (Sanjeevini) plant extract, *International Journal of Biological Macromolecule*, **2019**, 131(15), 787-797.
[9]. M. B. Marulasiddeshwara, S. S. Dakshayani, M. N. Sharath Kumar, R. Chethana, P. Raghavendra Kumar, S. Devaraja, Facile-one pot-green synthesis, antibacterial, antifungal, antioxidant and antiplatelet activities of lignin capped silver nanoparticles: A promising therapeutic agent, *Materials Science and Engineering C*, **2017**, 81,182-190**.**
[10]. Aparna Bhardwaj, Somendra Sharma Phytochemical and Antibacterial Studies on Methanolic Flower Extracts of *Nyctanthes arbor-tristis* L. *J. Applicable Chem.*, **2018**, 7(4), 958-963.
[11]. A. D. Olasupo, A. B. Aborisade O. V. Olagoke,Phytochemical Analysis and Antibacterial Activities of Spinach Leaf, *American Journal of Phytomedicine and Clinical*, **2018**, 6(2), 1-4.
[12]. D. Metodiewa, A .Kochman, S. Karolczak, Evidence for antiradical and antioxidant properties of four biologically active *N, N*, diethylaminoethyl ethers of flavanone oximes: a comparison with natural polyphenolic flavonoid (rutin) action, *Biochem Mol Biol Int.*, **1997**, 41,1067–1075.
[13]. T. Hayashi, K. Sawa, M. Kawasaki, Inhibition of cow's milk xanthine oxidase by flavonoids, *J Nat Prod.*, **1988**, 51, 345–348.
[14]. E. Walker, M. Pacold, O. Perisic, Structural determinations of phosphoinositide 3-kinase inhibition by wortmannin, LY294002, quercetin, myricetin, and staurosporine, *Mol Cell*, **2000**, 6, 909–919