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Screening and antibacterial activity analysis of some important medicinal plants

G. Senthilmurugan @ Viji, B. Vasanthe, and Kuru Suresh

Department of Botany,
Thiagarajar College of arts and science,
Madurai 09, Tamilnadu state, india

ABSTRACT: The screening and study of five different plant specimens belonging to different families for phytochemical constituents was performed using generally accepted laboratory technique for qualitative determinations. The constituents screened were saponins, combined anthraquinones, terpenoids, flavonoids, carotenoids, steroids, xantho proteins, couramins, alkaloids, quinones, vitamin C. The distribution of these constituents in the plant specimens were assessed and compared. The medicinal plant studied were Acalypha indica, Camellia sinensis, Plectranthus amboinicus, Curcuma longa, Rauvolfia tetraphylla. All the plant speciemens were found to contain terpenoids, xantho proteins, couramins and vitamin C. They also contain Saponins (except Curcuma longa), Combined anthroquinones (except Acalypha indica, Camellia sinensis, Curcuma longa) flavonoids (except Acalypha indica, Camellia sinensis), Carotenoids (except Acalypha indica, Curcuma longa), and steroids (except Plectranthus amboinicus, Rauvolfia tetraphylla) Quinones were found in one out of the five specimens. Some of the medicinal plant seemed to have potential as source of useful drugs. Though the one percent extracts of all the plants showed some degree of antimicrobial activity, it was significant in Acalypha indica, Camellia sinensis, Plectranthus amboinicus, Curcuma longa, and Rauvolfia tetraphylla. The extract of Camellia sinensis and Acalypha indica was most effective against Enterobacter faecalis (ZI = 3 cm and ZI = 1.7cm) and Camellia sinensis and Acalypha indica was most effective against Staphylococcus aureus (ZI = 2.1 cm).

KEYWORDS: Botany, Plant extracts, Phytochemical screening, Medicinal plants, Antibacterial activity.

1 INTRODUCTION

Medicinal plants are natural sources of compounds that can be used against many diseases today (Kubmarawa et al., 2007). Since a variety of plants grow in every conceivable place, having access to them would require only previous knowledge of their location and certain unique characteristics, such as a plant's habit of growth. As such, plants can be obtained easily. This aspect would be vital in discovering medicinal plants with high biological activity, low toxicity and which are acquired at most people in developing countries resort to local traditional medicine due to lack of doctors in their communities and their financial incapability in purchasing market-based medicine. Hence, extensive research on the use of cheaper plant-based therapy is imperative nowadays. Through this study, the importance of the plant biodiversity in the country was also highlighted as a result of the dependence on them for medicinal purposes. Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer et al., 1999). The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants in Hindu culture is founds in "Rigveda", which is said to have been written between 4500 - 1600 B.C. and is supposed to be the oldest repository of human knowledge. It is Ayurveda, the therapy involves the use of plant extract and their active constituents (Akerele, 1993).

About 1500 plants are systematically used in indigenous system of medicine, like Ayurveda, Unani and Siddha. However, the ethno pharmacologists, botanists, microbiologists and natural-product chemists world over today, is constantly still in search of medicinal efficacy of plants and their phytochemicals, since the reported data so far available on plants are

Corresponding Author: G. Senthilmurugan @ Viji (viji.m6@gmail.com)

comparatively meager before the vast number of plant population. The drugs which are already in use to treat infectious diseases is concern because, drug safety remains an enormous global issue. It was estimated that 2.22 million hospitalized patients had serious Adverse Drug Reactions (ADR) and 106,000 died in a single year in the USA. This Herbal and natural products have been used in folk medicine for centuries throughout the world, but there are relatively lower incidences of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, this coupled with their reduced cost, is encouraging for both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs (Nair et al., 2005). Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Mojab, Kamalinejad, Ghaderi and Vahidipour, 2003). Chemically constituents may be therapeutically active or inactive. The ones which are active are called active constituents and the inactive ones are called inert chemical constituents (Iyengar, 1995).

The principle aim of the present work was to study the screening and antibacterial activity of Acalypha indica, Camellia sinensis, Plectranthus amboinicus, Curcuma longa, Rauvolfia tetraphylla plant extracts in solvents like, absolute alcohol, most effective against both human and plant pathogenic bacteria including Enterobaceter faecalis, and Staphylococcus aureus.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

Fresh leaves of five different medicinal plants belonging to different families which are free from diseases were collected during the month of August to September, 2012 from Megamalai hills and different places of Manamadurai and Madurai. Taxonomic identification of the plants was carried out with the help of The Flora of Presidency of Madras (Gamble, 1935). The leaves of the medicinal plants Acalypha indica, Camellia sinensis, Plectranthus amboinicus, Curcuma longa, and Rauvolfia tetraphylla were used for the experiment.

2.2 IDENTIFICATION TESTS

The tests were done to find the presence of the active chemical constituents such as saponins, combined anthroquinones, terpenoids, flavonoids, carotenoids, steroids, xantho Proteins, couramins, alkaloids, quinones and vitamin c by the following procedure.

Test for Saponins: 1 ml of leaf extract was separately boiled with 2 ml of distilled water in a water bath for 10minutes. The mixture was filtered while hot and allowed to cool. 2.5 ml of filtrate was diluted to 10ml with distilled water and shaken vigorously for 2minutes (frothing indicated the presence of saponins in the filtrate).

Test for Combined Anthraquinones: 1 ml of sample was boiled with 2 ml of 10 % hydrochloric acid for 5 mins. The mixture was filtered while hot and filtrate was allowed to cool. The cooled filtrate was partitioned against equal volume of chloroform gets separated and forms a layer above the filtrate. The chloroform layer was transferred into a clean dry test tube using a clean pipette. Equal volume of 10 % ammonia solution was added into the chloroform containing test tube. It was shaken and allowed to separate. The separated aqueous layer was observed for any colour change; delicate rose pink colour showed the presence of an anthraquinones.

Test for Terpenoids: 1 ml of leaf extract was mixed with 0.5 ml of chloroform. Then 1.5 ml of concentrated H_2SO_4 was added to form a layer. A reddish brown precipitate coloration at the interface formed indicated the presence of terpenoids.

Test for Flavonoids: 1ml of leaf extract was boiled with 5 ml of distilled water for 5 minutes and filtered while hot. Few drops of 20 % sodium hydroxide solution were added to 1 ml of the cooled filtrate. It changes in to a yellow colour which on addition of acid changed to colourless solution depicted the presence of flavonoids.

Test for carotenoids: 1 ml of leaf extract was mixed with 5 ml of chloroform in a test tube. It was shaken vigorously. The resulting mixture was filtered and 85 % sulphuric acid was added. A blue colour at the interface showed the presence of carotenoids.

Test for Steriods: 1 ml of leaf extract was dissolved in 5 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

Test for Xantho Proteins: 1ml of leaf extract mixed with few drops of nitric acid then few drops of ammonia was added to its red colour formed. This indicated the presence of xantho proteins.

Coumarins: l ml of leaf extract was mixed with few drops of NaOH and 1 ml of alcohol was added formation of yellow colour indicates the presence of coumarins.

Alkaloids: Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloid solution produces white yellowish precipitate when a few drops of Mayer's reagents are added (Siddiqui and Ali, 1997). Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent (Evans, 2002).The alcoholic extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The samples were then observed for the presence of turbidity or green precipitation.

Quinones: 1 ml of leaf extract mixed with 2 ml of alcohol and 1 ml of KOH was added formation of blue colour indicates in the presence of quninones.

Test for Vitamin C: 1 ml of leaf extract add with 2ml of water was added. Then 0.1g of sodium bicarbonate and about 20mg of ferrous sulphate was added and shaked. Then it was allowed to for few minutes a deep violet colour is produced. Add 5ml of 1M sulphuric acid, the colour disappears.

2.3 ANTIBACTERIAL ASSAY

Nutrient Agar medium was prepared according to the manufacturer's instructions. The medium was sterilized by autoclaving at 121˚C for 15 minutes at 15 psi pressure and was used for tests. Sterile nutrient agar medium was poured aseptically into sterile petridishes (15 ml each) and the plates were allowed to solidify at room temperature in sterile condition. After solidification 1 ml of bacterial culture Enterobacter faecalis, staphylococcus aureus was placed on the medium. It was then spreaded over the surface of agar using 'L 'rod. Four wells were bored on the agar surface using a cork borer. One well was filled with sterile water and remaining three wells were filled with extract of plants such as Acalypha indica, Camellia sinensis, Plectranthus amboinicus, Curcuma longa, and Rauvolfia tetraphylla respectively. The next day the zones of inhibition were measured with a measuring scale. This experiment was carried out in triplicate for their confirmation. The results were read by the presence or absence of zone of inhibition.

3 RESULTS

Table 1. is the scientific, family, English and local names of the medicinal plants that were screened for phytochemical constituents.The screening of these five different medicinal plant species namely Acalypha indica, Camellia sinensis, Plectranthus amboinicus ,Curcuma longa and Rauvolfia tetraphylla for phytochemical constituent was performed using generally accepted laboratory technique for qualitative determinations.

Table 2. It should be noted that steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones. The study indicated that terpenoids, xantho proteins, couramin and vitamin c compounds were present in all the medicinal plants They also contain Saponins (except Curcuma longa), Combined anthroquinones (except Acalypha indica, Camellia sinensis, Curcuma longa) flavonoids (except Acalypha indica, Camellia sinensis), Carotenoids (except Acalypha indica, Curcuma longa), and steroids (except Plectranthus amboinicus, Rauvolfia tetraphylla) Quinones were found in one plant out of the five specimens.

Table 3. Though the one percent extracts of all the plants showed some degree of antibacteriall activity, it was significant in Acalypha indica, Camellia sinensis, Plectranthus amboinicus, Curcuma longa, and Rauvolfia tetraphylla. The extract of Camellia sinensis and Acalypha indica was most effective against Enterobacter faecalis (ZI = 3 cm and ZI = 1.8 cm) and Camellia sinensis and Acalypha indica was most effective against Staphylococcus aureus (ZI = 2.1 cm and ZI = 1.7 cm). Green tea leaves and extracts have shown to be effective against bacteria responsible for bad breath. The tea component Epicatechin gallate is being researched because in vitro experiments showed it can reverse Methicillin resistance in bacteria like Staphylococcus aureus. Zone of inhibition of the individual plant extracts are shown.

4 TABLES AND FIGURES

4.1 TABLES

Table 1. Scientific, Family, English and Local names of the medicinal plants investigated

Scientific Name	Family Name	English Name	Local Name
Acalypha indica L.	Euphorbiaceae	Indian nettle	Kuppaimeni
Camellia sinensis (L.) Kuntze	Theaceae	Tea	Theilai
Plectranthus amboinicus (Lour.) Spreng.	Lamiaceae	Country borage	karpuravalli
Curcuma longa Linnaeus.	Zingiberaceae	Turmeric	Manjal
Rauvolfia tetraphylla L.	Apocynaceae	Wild snake root	Pampukaalaachchedi

Table 2. Result of the Phytochemical screening of the medicinal plants

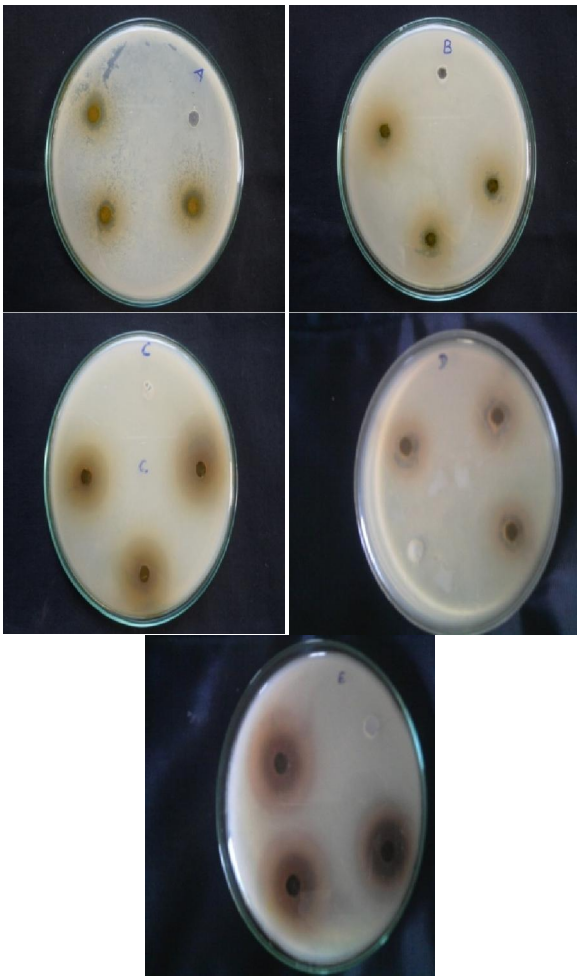
Plant name	Sap	Com Anth	Ter	Fla	Car	Ste	Xan pro	Cou	Alk	qui	Vit c
Acalypha indica L.	+	-	+	-	-	+	+	+	+	-	+
Camellia sinensis (L.) Kuntze	+	-	+	-	+	+	+	+	+	-	+
Plectranthus amboinicus (Lour.) Spreng.	+	+	+	+	+	-	+	+	+	-	+
Curcuma longa Linnaeus.	-	-	+	+	-	+	+	+	+	+	+
Rauvolfia tetraphylla L.	+	+	+	+	+	-	+	+	+	-	+

a). Sap= saponins b). Com Anth= combined anthroquinones c). Ter= Terpenoids d). Fla= Flavonoids e). Car= Carotenoids, f). Ste= Steroids, g). Xan pro= Xantho proteins, h).Cou= Couramin i). Alk= Alkaloids j). Qui= Quinones k). Vit c.= Vitamin c, + = present, - = Absent.

Table 3. Zone of inhibition of individual plant extracts

Bacteria	Plants	1 % plant extract (Zone inhibition cm)
Enterobacter faecalis	Acalypha indica	1.8 cm
	Camellia sinensis	3 cm
	Curcuma longa	1.2 cm
	Plectranthus amboinicus	1.3 cm
	Rauvolfia tetraphylla.	1.3 cm
Staphylococcus aureus	Acalypha indica	1.7 cm
	Camellia sinensis	2.1 cm
	Curcuma longa	1.2 cm
	Plectranthus amboinicus	1.4 cm
	Rauvolfia tetraphylla.	1.5 cm

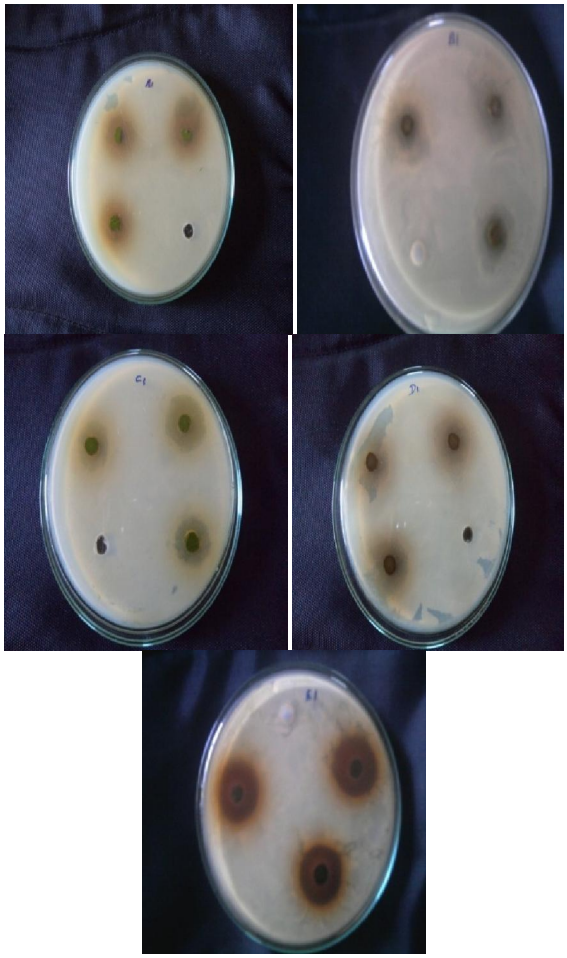
4.2 FIGURES



A=Curcuma longa, B=Rauvolfia tetraphylla, C=Acalypha indica, D=Plectranthus amboinicus, E=Camellia sinensis.

Fig. 1.Inhibition zone of five different family plant extract against Enterobacter faecalis

Fig. 1 shows the five different plant extract against Enterobacter faecalis



A=Curcuma longa, B=Rauvolfia tetraphylla, C=Acalypha indica, D=Plectranthus amboinicus, E=Camellia sinensis.

Fig. 2.Inhibition zone of five different family plant extract against Staphylococcus aureus.

Fig. 2 shows the five different plant extract against Staphylococcus aureus

5 CONCLUSION

The ethanolic extracts of the studied plants contained many bioactive chemical constituents including saponins, combined anthroquninones, terpenoidS, flavonoidS, carotenoids, steroids, xantho Proteins, couramins, alkaloids, quinones and vitamin c. The extract of Camellia sinensis and Acalypha indica was most effective against Enterobacter faecalis (ZI = 3 cm and ZI = 1.7 cm) and Camellia sinensis and Acalypha indica was most effective against Staphylococcus aureus (ZI = 2.1 cm and 1.7 cm).

REFERENCES

[1] A.A. Siddiqui, M. Ali, "Practical Pharmaceutical chemistry," 1st ed., CBS Publishers and Distributors, New Delhi, pp 126-131, 1997.
[2] D. Kubmarawa, GA. Ajoku, NM. Enwerem, DA. Okorie, "Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria," *Afr. J. Biotechnol*. 6: 1690-1696, 2007.
[3] F. Mojab, M Kamalinejad, N. Ghaderi, H. Vahidipour, "Phytochemical Screening of Some Iranian Plants," *Iranian Journal of Pharmaceutical Research*. pp 77-82, 2003.
[4] J. S. Gamble, "The Flora of the Presidency of Madras," London: Adlard & Son, Ltd. London, 1935.
[5] KA. Hammer, CF. Carson, TV. Riley, "Antimicrobial activity of essential oils and other plant extracts*," J. Appl. Microbiol*., 86(6): 985, 1999.
[6] MA. Iyengar, "Study of Crude Drugs," 8th ed., Manipal Power Press, Manipal, India. pp. 2, 1995.
[7] O. Akerele, "Summary of WHO Guidelines for the Assessment of Herbal Medicines Herbal Gram," 22: 13-28, 1993.
[8] R. Nair, T. Kalariya, S. Chanda, "Antibacterial activity of some selected Indian medicinal flora," Turk. *J. Biol*., 29: 41-47, 2005.
[9] WC. Evans, "Trease and Evan's Pharmacognosy," 5th ed. Haarcourt Brace And Company, pp. 336, 2002.