

COMPREHENSIVE STUDY ON PHYTOCHEMICAL ANALYSIS OF MEDICINAL PLANTS IN INDIA

A. Rajasekhar Babu, P. Dinesh Sankar Reddy*

Department of Chemical Engineering, JNTUA College of Engineering,
Ananthapuramu, India

ABSTRACT

Medicinal plants have biological compounds which are used for treatment of various human diseases and also play an important role in healing. The traditional medicine involves the use of different plant extracts or the bioactive constituents. Phytochemicals have two categories i.e., primary and secondary constituents. Primary constituents have chlorophyll, proteins sugar and amino acids. Secondary constituents contain flavonoids, terpenoids, phlobatannins, phenols and alkaloids... etc. Medicinal plants have antifungal, antibacterial and anti-inflammation activities.

The present study involves different medicinal plants like Phyllanthus emblica(amlam), Eucalyptus globulus(eucalyptus), Musa(banana), Hibiscus rosa-sinensis(hibiscus), Ocimum tenuiflorum(tulasi), Murraya koenigii(curry), menthe(mint), Piper betle(betel), Moringa oleifera(drumstick), and Tamarindus indica(tamarind) for phytochemical analysis. The leaves of the selected medicinal plants were washed, shadow dried and then powdered. The aqueous extract of leaf samples were used for the various phytochemical tests to find out the phytochemical constituents present in the plants. The main objective of the work was to check the presence or absence of the phytochemical constituents in all the selected medicinal plants.

The results of the phytochemical analysis of these medicinal plants showed that flavonoids, terpenoids, phlobatannins, phenols and alkaloids are found to be present in above mentioned medicinal plants. The phytochemical analysis of the plants is very important commercially and has great interest in pharmaceutical companies for the production of the new drugs for curing of various diseases.

Keywords: Alkaloids, Antibacterial, Medicinal plants, Phytochemicals, Phenols.

I. INTRODUCTION

In India, as we all are aware of the herbal medicine treatment which is taking place for almost centuries. We have a wide variety of flora and fauna which are not only used for the decoration purposes but they were used in the treatment of so many chronic diseases. One can think in the way that what is the necessity of going for herbal treatment where we are having allopathic treatment which is quick enough in curing diseases. But the problem with the allopathic treatment arises in the cases of chronic diseases where the toxicity of the chemicals will make the patient to go through so many side effects and even to death in some cases. The herbal plants which are having variety of properties like antioxidant [1,2], antiulcer [3,4], antifungal [5], antibacterial [6], anti

analgesic, anti-inflammatory [7], anticancer [8]...etc. In the case of herbal treatment there will be no side effects when compared with other type of treatments as the herbs are derived from natural resources which are easily available in India. So it is evident that the herbal medicinal treatment is very much useful in curing the chronic diseases.

In the present work a study on Phytochemical analysis for various plants like *Phyllanthus emblica* (amla) [9], *Eucalyptus globulus*(eucalyptus) [10,11], *Musa*(banana) [12], *Hibiscus rosa-sinensis*(hibiscus) [13], *Ocimum tenuiflorum*(tulasi) [14], *Murraya koenigii*(curry), *menthe*(mint) [15], *Piper betle*(betel) [16,17,18,19], *Moringa oleifera*(drumstick), and *Tamarindus indica*(tamarind) [20, 21, 22, 23] used in the synthesis of nanoparticles is performed. Phytochemicals usually consists of primary and secondary metabolites, of which the present study is concentrated on the study of secondary metabolites which exhibit good medicinal properties. Some of the most common secondary metabolites having medicinal properties are flavanoids, alkaloids, anthraquinones, reduced sugars, phlobatannins, phenols, steroids, carbohydrates, amino acids, proteins, fixed oils and fats, phytoserols, tannins, glycosides, saponnins, cardiac glycosides, terpenoids and cardenoids. This type of studies improves the evolution of new medicines for curing many diseases which makes the availability of medicines to common man affordable.

Determination of the presence of secondary metabolites in plant extracts paves a new path for green synthesis of many metal and semiconductor nanoparticles, thus reducing the load on the use of synthetic reducing agents. The nanoparticles generated using these plant extracts has shown the presence of the aforementioned metabolites as a capping agent and has exhibited good medicinal applications.

II. MATERIALS AND METHODS

2.1. Plant Material and extraction:

Leaves of the selected medicinal plants which are mentioned above are collected from Ananthapuramu region washed with tap water to remove dust particles again rinsed with double distilled water to remove any contaminants and then the leaves were shadow dried individually for 15 days at room temperature and are powdered using a mini ball mill. The powdered samples are stored in a container for extraction. To prepare plant extract, 50 grams of the prepared powder from each selected plant is individually placed in 500ml conical flasks by adding 250ml of double distilled water and then boiled at 50⁰-60⁰c in a water bath for 30 minutes. After 30 minutes, the mixture is cooled and then filtered using whatmann grade1 filter paper. After filtration the liquid extract collected is stored at 4⁰c in a refrigerator for further Phytochemical analysis.

2.2. Phytochemical Analysis:

The preliminary Phytochemical tests were performed for testing the presence or absence of different Phytochemical constituents like flavanoids, alkaloids, antraquinones, reduced sugars, phlobatannins, steroids, carbohydrates, amino acids, fixed oils and fats, phytoserols, tannins, glycosides, terpenoids, saponnins, cardiac glycosides and cardenoids in the selected medicinal plants through positive reaction with respective test reagent.

For Flavonoids: 2ml of dilute NAOH was added to 2 ml of extract the appearance of yellow color indicates the presence of flavanoids.

For Terpenoids: 0.5ml of acetic anhydride was mixed with 1 ml of sample extract and a few drops of concentrated H_2SO_4 ; a bluish green precipitate indicates the presence of terpenoids.

For Anthraquinones: 0.5 g of extract was taken with 10 ml of ammonia solution was added to the filtrate and the mixture was shaken the formation pink, red or violet colour on Ammonical phase indicates the presence of Anthraquinones.

For Phlobatannins: A few drops of 1% HCL was added to 1 ml of extract and boiled a red precipitate indicates the presence of phlobatannins.

For Saponins: 1 ml of distilled water was added to 1ml of extract and shaken vigorously .a stable persistent froth indicates the presence of saponins

For Phenols: Equal volumes (1ml) of extract and iron chloride was mixed a deep bluish green solution gave the indication of the presence of phenols

For Tannins: A portion of extract was dissolved in water after which the solution was filtered and 10% of ferric chloride solution was then added to the resulting filtrate the appearance of bluish black colour indicates the presence of tannins

For Fixed Oils and Fats: A small quantity of extract was pressed between two filter papers .oil strain on the paper indicates the presence of fixed oils

For Alkaloids: To a few ml of filtrate, a drop of Mayer's reagent was added by the side of the test tube is white creamy precipitate indicate the test as positive (Mayer's test).

For Carbohydrates: To 2ml of plant extract, two drops of alcoholic solution of alpha Naphthol solution and conc. H_2SO_4 was added violet ring indicates the presence of carbohydrates (Molisch's Test).

For Proteins and Amino Acids: Two drops of Ninhydrin solution was added to 2ml of plant extract, development of pink colour indicates the presence of proteins and amino acids (Nin Hydrin Test).

For Glycosides: To 2ml of conc. Sample (leaf extract), add 20ml of 50% H_2SO_4 and heated in the water bath for 15 minutes a 10ml of Fehling's solution added to the mixture and boiled, development of brick red colour precipitate indicate the presence of glycosides

For Reducing Sugars: To the extract 5ml, distilled water is added and boiled in the presence of Fehling solution A and B, orange red precipitate indicates the presence of reducing sugars

For Steroids: To 2ml of filtrate, add 2ml of acetic anhydride and conc. H_2SO_4 , blue green ring and red ring indicates the presence of steroids

For Phytosterols: To the extract 2ml of acetic anhydride and 1 to 2 drops of H_2SO_4 is added, an array of colour change indicates the presence of phytosterols (Liebermann-Burchards Test).

For Cardenoids: 2ml of benzene is added to 1ml of the sample extract the formation of turbid brown colour indicates the presence of cardenoids.

For Cardiac Glycosides: Keller-kiliani test was performed for checking the presence of cardiac glycosides. The crude powder of flower was treated with 1.0 ml mixture of 5% $FeCl_3$ and glacial acetic acid (1:99v v-1). To this solution, a few drops of concentrated H_2SO_4 were added. Appearance of greenish blue colour within few minutes indicated the presence of cardiac glycosides.

III. RESULTS AND DISCUSSION

After performing the various test listed in the previous section, the results which represent the presence or absence of the secondary metabolites are provided in Tables 1 to 4. ‘+’ and ‘-’ signs in these tables indicate the presence and the absence of phytochemicals in each plant extract respectively. The data shown in Tables 1, 2, 3 & 4 shows screening of aqueous extracts of leaves of ten medicinal plants viz., Amla (*Phyllanthus Emblica*), Eucalyptus (*Eucalyptus Globulus*), Banana (*Musa Acuminata*), Hibiscus (*Hibiscus Rosa-Sinensis*), Tulasi (*Ocimum Tenuiflorum*), Curry (*Murraya Koeniggi Spreng*), Mint (*Mentha X Rotundifolia(L) Huds*), Betel (*Pipper Betle*), Drumstick (*Moringa Oleifera*), Tamarind (*Tamarindous Indica*) based on phytochemical tests. Table.1 depicts the presence/absence of steroids, carbohydrates, amino acids and anthraquinones, Table.2 depicts the presence of phytosterols, tannins, glycosides and reduced sugars, Table.3 depicts the presence of alkaloids, flavanoids, terpenoids and saponins and Table.4 depicts the presence of fixed oils and fats, phlobatannins, cardiac glycosides and cardenoids.

S.NO	PLANT SPECIES	STEROIDS	CARBOHYDRATES	AMINO ACIDS AND PROTEINS	ANTHRAQUINONES
1	AMLA (<i>Phyllanthus emblica</i>)	-	+	-	-
2	EUCALYPTUS (<i>Eucalyptus globulus</i>)	+	+	-	-
3	BANANA (<i>Musa acuminata</i>)	+	+	-	-
4	HIBISCUS (<i>Hibiscus rosa-sinensis</i>)	-	-	+	-
5	TULASI (<i>Ocimum tenuiflorum</i>)	+	+	+	-
6	CURRY (<i>Murraya koeniggi spreng</i>)	+	+	+	+
7	MINT (<i>Mentharotundifolia(L)huds</i>)	-	+	-	-
8	BETEL (<i>Pipper betle</i>)	+	+	-	-
9	DRUMSTICK (<i>Moringa oleifera</i>)	+	+	+	-
10	TAMARIND (<i>Tamarindous indica</i>)	+	+	-	-

TABLE 1: Phytochemical analysis results for steroids, carbohydrates, amino acids & proteins and anthraquinones

From the results it is observed that the metabolites like flavanoids, saponins, glycosides, tannins, reducing sugars and carbohydrates are present in all the extracts of the plants considered. Whereas, plants like *Ocimum tenuiflorum* (Tulsi) and *Murraya koeniggi spreng* (Curry Leaves) have shown the presence of fifteen metabolites out of the sixteen metabolites considered in this study, thus making them suitable for many medicinal applications. Among the remaining plant extracts, *Mentharotundifolia(L)huds* (Mint) and *Tamarindous indica*

(Tamarind) have shown the presence of twelve and eleven metabolites thus making them equally suitable for many suitable medicinal applications.

S.NO	PLANT SPECIES	PHYTOSEROLS	TANNINS	GLYCOSIDES	REDUCING SUGARS
1	AMLA (<i>Phyllanthus emblica</i>)	-	+	+	+
2	EUCALYPTUS (<i>Eucalyptus globulus</i>)	+	+	+	+
3	BANANA (<i>Musa acuminata</i>)	+	+	+	+
4	HIBISCUS (<i>Hibiscus rosa-sinensis</i>)	+	+	+	+
5	TULASI (<i>Ocimum tenuiflorum</i>)	+	+	+	+
6	CURRY (<i>Murraya koenigispreng</i>)	+	+	+	+
7	MINT (<i>Mentharotundifolia(L)huds</i>)	+	+	+	+
8	BETEL (<i>Pipper betle</i>)	-	+	+	+
9	DRUMSTICK (<i>Moringa oleifera</i>)	-	+	+	-
10	TAMARIND (<i>Tamarindous indica</i>)	+	+	+	+

TABLE 2: Phytochemical analysis results for phytoserols, tannins, glycosides, reducing sugars

S.NO	PLANT SPECIES	ALKALOIDS	FLAVANOIDS	TERPENOIDS	SAPONINS
1	AMLA (<i>Phyllanthus emblica</i>)	+	+	+	+
2	EUCALYPTUS (<i>Eucalyptus globulus</i>)	+	+	+	+
3	BANANA (<i>Musa acuminata</i>)	+	+	-	+
4	HIBISCUS (<i>Hibiscus rosa-sinensis</i>)	+	+	-	+
5	TULASI (<i>Ocimum tenuiflorum</i>)	+	+	+	+
6	CURRY (<i>Murraya koenigispreng</i>)	+	+	+	+
7	MINT (<i>Mentharotundifolia(L)huds</i>)	+	+	+	+
8	BETEL (<i>Pipper betle</i>)	-	+	+	+
9	DRUMSTICK (<i>Moringa oleifera</i>)	-	+	+	+
10	TAMARIND (<i>Tamarindous indica</i>)	+	+	-	+

TABLE 3: Phytochemical analysis results for Alkaloids, flavanoids, terpenoids and saponins

S.NO	PLANT SPECIES	FIXED OILS AND FATS	PHLOBOTANNINS	CARDIAC GLYCOSIDES	CARDENOIDS
1	AMLA (<i>Phyllanthus emblica</i>)	-	-	+	-
2	EUCALYPTUS (<i>Eucalyptus globulus</i>)	+	-	+	-
3	BANANA (<i>Musa acuminata</i>)	+	-	+	+
4	HIBISCUS (<i>Hibiscus rosa-sinensis</i>)	+	-	+	+
5	TULASI (<i>Ocimum tenuiflorum</i>)	+	+	+	+
6	CURRY (<i>Murraya koenigisprengr</i>)	+	-	+	+
7	MINT (<i>Mentharotundifolia(L)huds</i>)	+	-	+	+
8	BETEL (<i>Pipper betle</i>)	-	+	-	+
9	DRUMSTICK (<i>Moringa oleifera</i>)	-	-	-	+
10	TAMARIND (<i>Tamarindous indica</i>)	+	-	+	-

TABLE 4: Phytochemical analysis results for fixed oils and fats, phlobatannins, cardiac glycosides and cardenoids

IV. CONCLUSIONS

These various phytochemical tests performed reveal the presence of variety of bioactive secondary metabolites which might be responsible for their medicinal attributes. Only four plants, *Ocimum tenuiflorum* (Tulsi), *Murraya koenigisprengr* (Curry Leaves), *Mentharotundifolia(L)huds* (Mint) and *Tamarindous indica* (Tamarind) have exhibited presence of many secondary metabolites compared to other plant extracts considered. This in turn gives an indication that, based on the bioactivity of the phytochemicals, the concerned plant species can be used for synthesis of nanoparticles which further can find applications in the treatment of various chronic ailments. From this study it is evident that few of the plant species which are available in Anantapuramu region can be used for effective medicinal applications.

REFERENCES

- [1] Kumar RV, Kumar S, Shashidhara S, Anitha S, Manjula M. Antioxidant and antimicrobial activities of various extracts of *Michelia champaca* Linn flowers. *World Appl Sci J* 2011; 12(4):413-418.
- [2] Kaneria M, Bapodara M, Chanda S. Effect of extraction techniques and solvents on antioxidant activity of Pomegranate (*Punica granatum L.*) leaf and stem. *Food Anal Method* 2012; 5:369-404.

- [3] Chanda S, Baravalia Y, Kaneria M. Protective effect of *Polyalthia longifolia* var. *pendula* leaves on ethanol and ethanol/HCl induced ulcer in rats and its antimicrobial potency. *Asian Pac J Trop Med* 2011; 4(9):673-679.
- [4] Kalaivani M, Jegadeesan M. Evaluation of antiulcer activity of ethanolic extract of *Madhuca longifolia* flowers in experimental rats. *Int J Sci Res Publication* 2013;3(6):1-7.
- [5] Maneemegalai S, Naveen T. Evaluation of antibacterial activity of flower extracts of *Cassia auriculata*. *EthnobotLeaf lets* 2010; 14:8-20.
- [6] Menpara D, Desai D, Rathod T, Chanda S. Evaluation of nutraceutical bottle gourd (*Lagenaria siceraria*) as a potential source of natural antimicrobial agent. *Am J Clin Therapeut* 2014; 2(3):375-389.
- [7] Tanna A, Nair R, Chanda S. Assessment of anti-inflammatory and hepatoprotective potency of *Polyalthia longifolia* var. *pendula* leaf in Wistar albino rats. *J Nat Med* 2009; 63:80-85.
- [8] Thirumal M, Kishore G, Prithika R, Das S, Nithya G. In vitro anticancer activity of *Tecoma stans* (L.) ethanolic leaf extract on human breast cancer cell line. *Int J Pharmaceut Chem Biol Sci* 2012; 2(4):488-493.
- [9] Pandya G. and S.P. Pandya (2011). Phytochemical and toxicity study of *Emblca officinalis* (Amla). *International Research Journal Of Pharmacy* 2(3): 270-272.
- [10] Reena Saxena, PramodPatil and S.S. Khan Screening for phytochemical analysis of *Eucalyptus globulus* Labill. and *Emblca officinalis*. *Gaertn Nanobiotechnica Universale* Vol. 1(2), 103-106 (2010).
- [11] Egwaikhide, P.A., S.O. Okeniyi, E.E. Akporhonor and S.A Emua (2008). Studies on Bioactive metabolites constituents and antimicrobial Evaluation of leaf Extracts of *Eucalyptus globules*. *Journal of Agricultural* 3(1): 42-45.
- [12] DR. N. Gunavathy, DR. S. Padmavathy, DR. S.C. Murugavel Phytochemical screening of *Musa Acuminata* leaf extract, *Impact Factor* 1.393, ISSN: 2320-5083, Volume 2, Issue 1, February 2014
- [13] Manish Kumar, Rajneesh Garg and Rakesh Garg, Phytochemical Properties and Antioxidant Activity of *Hibiscus sabdariffa* Linn, *International journal of Pharmaceutical and chemical sciences*, ISSN: 22775005, Vol. 1 (3) Jul-Sep 2012.
- [14] Choudhury Golak Bihari, Behera Manaswini, Jena Prabhat Kumar and Tripathy Sujit Kumar. Pharmacognostical and phytochemical investigation of various tulasi plants available in south eastern Odisha, *International Journal of Research in Pharmaceutical and Biomedical Sciences*, ISSN: 2229-3701.
- [15] Arumugam P, Ramamurthy P, Santhiya ST, Ramesh A. Antioxidant activity measured in different solvent fractions obtained from *Menthaspicata* Linn: An analysis by ABTS. + decolorization assay. *Asia Pac J Clin Nutr.* 2006; 15(1):119124.
- [16] O.Sita kumari¹, Dr. Nirmala Babu Rao². Phytochemical analysis of *Piper betel* leaf extract. *World Journal of Pharmacy and Pharmaceutical Sciences*, ISSN 2278 – 4357, Volume 4, Issue 1, 699-703.
- [17] Sarkar A, Sen R, Saha P, Ganguly S, Mandal G, Chatterjee M (2008). An ethanolic extract of leaves of *Piper betle* (Paan) Linn mediates its antileishmanial activity via apoptosis. *Parasitol. Res*, 2008; 102(6): 1249-55.

- [18] Chaurasia, Sundeep; Kulkarni, GirirajTirupatirao; Shetty, Laxmi Narayan; Mishra, Brahmeshwar; Phytochemical Studies and In vitro Cytotoxicity Screening of Piper betle Leaves Extracts; Journal of Pharmacy Research, Nov 2011; 4(11): 4187.
- [19] AraniDatta, Shreya Ghoshdastidar and Mukesh Singh*; Antimicrobial Property of Piper betel Leaf against Clinical Isolates of Bacteria; Journal of Pharma Sciences and Research (IJPSR), 2011; 2(3): 104-109.
- [20] Gupta, C., 1Prakash, D. and 2Gupta, S.Studies on the antimicrobial activity of Tamarind (Tamarindusindica) and its potential as food bio-preservative, International Food Research Journal 21(6): 2437-2441 (2014).
- [21] Doughari,J.H.. Antimicrobial activity of Tamarindusindica Linn. Tropical Journal of Pharmaceutical Research, 2006, 5: 597-603.
- [22] Escalona-Arranz, J.C., Peres-Roses, R., UrdanetaLaffita, I., Camacho-Pozo, M.I., Rodrigues-Amado, J. and Licea-Jiminez, I. 2010. Antimicrobial activity of extracts from Tamarindusindica L. leaves. Pharmacognosy Magazine 6: 242-247.
- [23] Satish A Bhalerao, Deepa R Verma, Rohan V Gavankar, Nikhil C Teli, Yatin Y Rane,Vinodkumar S Didwana and Ashwin Trikannad; Phytochemistry, Pharmacological profile and Therapeutic uses of Piper Betle Linn. Research and Reviews: Journal of Pharmacognosy and Phytochemistry, 2013.