World Journal of Science and Research. 3(1): 30-36 (2018)

ISSN: 2455 2208

Available online at http://www.harmanpublications.com

World Journal of Science and Research

Research Article Botany

SCREENING OF PHYTOCHEMICALS AND ANTIMICROBIAL ACTIVITY OF *Erythrina indica* Lam.

A. Deepa and R. Sagaya Giri *

PG and Research Department Of Botany, Kunthavai Naacchiyaar Govt.Arts College For Women (Autonomous) Thanjavur – 613 007, Tamilnadu

ABSTRACT

In the present study was carried out to screen the phytochemicals, vitamins, histochemicals, Fluorescence and UV-Visible analysis and antimicrobial activity of *Erythrina indica* leaves extract. The phytochemical screening of *Erythrina indica* leaves showed that the presence of tannin, saponins, flavonoids, protein, glycosides, terpenoids, steroids, polyphenol, triterpenoids, carbohydrate and anthroquinones whereas phlobatannins was absent in methanol and aqueous extracts. Alkaloids were present only methanol extract. Quantitative analysis revealed that the plant has phenol (77.60mg/gm), flavonoids (110mg/gm) and terpenoids (9mg/gm)were presented. The vitamin analysis of *Erythrina indica* leaves showed that the presence of vitamin E whereas vitamin A, C and D were absent. The histochemical analysis further confirmed in the presence of flavonoids, alkaloids, glycosides, terpenoids, steroids, polyphenol, tannin and saponin. The fluorescence behavior of leaves powder proved by this study. The result of UV-VIS spectroscopic analysis confirms the presence of phenolic compounds in the *Erythrina indica* leaves extract. The results reveal that extract of *Erythrina indica* leaves were significantly effective against bacteria species *E. coli* and fungi species *C. albicans*

***Citation*:** A. Deepa and R. Sagaya Giri. (2018). Screening of phytochemicals and antimicrobial activity of *Erythrina indica* Lam. *World Journal of Science and Research*. 3(1): 30-36.

Article Info:

Received on 10st Feb 2018
Accepted on 20th March 2018
Online March 2018

Keywords:
Erythrina indica
Fluorescence behavior
Phytochemical,
antimicrobial activity
,

****Corresponding author***
Dr. R. Sagaya Giri,
Assistant Professor
PG and Research Department Of Botany, Kunthavai Naacchiyaar Govt.Arts College For Women (Autonomous) Thanjavur – 613 007, Tamilnadu

INTRODUCTION

Phytochemicals are not essential nutrients and are not required by the human body for sustaining life, but have important properties to prevent or to fight some common diseases. Many of these benefits suggest a possible role for phytochemicals in the prevention and treatment of disease, Because of this property; many researchers have been performed to reveal the beneficial health effects of phytochemicals. The purpose of the present review is to provide an overview of the extremely diverse phytochemicals presents in medicinal plants. Plant and plant products play a wide range of biological properties. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. Keeping in view, the present study was carried out to investigate the phytochemical analysis of *Erythrina indica* leaves.

MATERIALS AND METHODS

Collection of plant materials

The leaves of *Erythrina indica* Lam. were collected in December 2017 from Thanjavur, Tamil Nadu, India. The *Erythrina indica* leaves were washed several times with distilled water to remove the traces of impurities from the leaves. Leaves was spread out in a plain paper and shade dried at room temperature for about 10 days and makes a fine powder using grinder mixture. The powder materials were used for further studies.

Preparation of plant extract:

2 gram of the powder of *Erythrina indica* leaves were transferred in to different conical flask (250ml). The conical flask containing 50ml of different solution (methanol and water). The conical flask containing *Erythrina indica* leaves were shakeit well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using whatman filter paper No.1 and filtrate was used for further analysis.

Phytochemical screening

Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973 and 1984).

Quantitative analysis of phytochemicals

Total phenols estimated by the method of Edeoga *et al*., (2005). Flavonoid determine by the method of Bohm and Kocipai-Abyazan (1994). Total terpenoid content in the leaf extracts were assessed by standard method (Ferguson, 1956). Qualitative Analysis of Vitamins (Pearson, 1976; Patel, 2005). Determination of Fluorescence behavior of plant powder (Rao *et al.*, 2011)

Histochemical tests

A small quantity of dried and finely powdered leaves sample was placed on a grease free microscopic slide and treated with specific chemicals and reagents and waited for 1-2 minutes. A positive test for histochemicals was indicated by the appearance of the appropriate colour change after application of the reagent. Using a light microscope to observe and record any colour changes. The leaf powder treated with diluted ammonia and H_2SO_4 gave yellow colour indicates flavonoids. Plant powder treated with ferric chloride to give Dark blue to black indicates the presence of tannin. Plant powder treated with H_2SO_4 (few drops) to give yellow colour indicates the presence of Saponin.

UV-Visible analysis

The extracts were examined under visible UV-Visible spectrum. The sample is dissolved in same solvent. The extracts were scanned in the wavelength ranging from 340-960 nm using Systronic Spectrophotometer. These solutions were scanned in turn at intervals of 10 nm and the characteristic peaks were detected. The peak value of the UV-Visible was recorded.

Determination of antimicrobial activity

The antimicrobial activity was performed by disc diffusion method. Antibiogram was done by disc diffusion method (NCCLS, 1993; Awoyinka *et al.*, 2007) using plant extracts. Petri plates were prepared by pouring 30 ml of NA /PDA medium for bacteria/fungi. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria/fungi from a broth culture. A sterile cotton swab is dipped into a standardized bacterial/ fungi test suspension and used to evenly inoculate the entire surface of the Nutrient agar/PDA plate. Briefly, inoculums containing bacteria specie were spread on Nutrient agar plates and fungus strains were spread on potato dextrose agar. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude extracts (50µl) were laid down on the surface of inoculated agar plate. The plates were incubated at 37ºC for 24 h for the bacteria and at room temperature (30±1) for 24-48 hr. for yeasts strains. Each sample was tested in triplicate. The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the samples were measured using a millimeter scale.

RESULTS AND DISCUSSION

The present study was carried out on the *Erythrina indica* leaves revealed the presence of medicinally active constituents. The phytochemicals of the *Erythrina indica* leaves was investigated and summarized in Table-1 and Plate 2 and 3.The phytochemical screening *Erythrina indica* leaves showed that the presence of tannin, saponins,flavonoids, protein, glycosides, terpenoids, steroids, polyphenol, triterpenoids, carbohydrate and anthroquinones whereas phlobatannins was absent in methanol and aqueous extracts. Alkaloids were present only in methanol extract.

Hassain *et al.*, (2011) screened phytochemical constituents from methanol leaf extract of *Bombax malabaricum*. Various organic 11 solvent extracts of *Pedalium murex* were subjected to preliminary phytochemical screenings by Thamizh mozhi et al. (2011). Selected 53 traditionally used medicinal plants from western region of India for their qualitative phytochemical screenings, total phenol and flavonoids contents. Pascaline et al. (2011) screened phytochemical constituents of some medicinal plants used by the Nandis of South Nandi District, Kenya.

Reena Ganesan *et al*., (2013) aimed to carry out preliminary phytochemical of six different solvents extracts from leaf and leaf derived callus of *Sebastiania chamaelea*. The preliminary phytochemical analysis reflects the presence of phenolic compounds, carbohydrate, alkaloids, phytosterols, fats and oils, terpenoids. The result highlights among two extracts, leaf extract show negligible activity than callus extracts

Kumar *et al*., (2013) investigated the preliminary phytochemical screening of the leaves of the plant *Lasia spinosa* (Lour) Thwaites. The phytochemical screening showed that the methanol and aqueous extracts contained alkaloid, the carbohydrates and the phenolic compounds were present in all of the solvent extract except petroleum ether extract. The chloroform, ethyl acetate and the aqueous extract contained glycosides whereas the saponins present in methanol and aqueous extract. The ethyl acetate extract contain only the flavonoids.

Quantitative analysis

Quantitative analysis revealed that the *Erythrina indica* leaves has phenol, flavonoids andterpenoids. Significant amount of phenol (77.60mg/gm), flavonoids (110mg/gm) and terpenoids (9mg/gm) were presented (Table 2). The above phytoconstituents were tested as per the standard methods.

The secondary metabolites formed also are an important trait for our food plants (taste, colour, scent, etc.) and ornamental plants. Moreover, numerous plant secondary metabolites such as flavonoids, alkaloids, tannins, saponins, steroids, anthocyanins, terpenoids, rotenoids etc. have found commercial application as drug, dye, flavour, fragrance, insecticide, etc. Such fine chemicals are extracted and purified from plant materials (Das *et al.*, 2010).

Leo Stanley *et al.*, (2011) reported that leaves of *C. pedata* showed the presence of alkaloids, carbohydrates, steroids, tannin, phenolic compounds, flavonoids and terpenoids. Dinesh kumar *et al.*, (2011) has been reported to terpenoids, flavonoids and tannin are present in *C. trifolia.* Rajmohanan *et al.*, (2014) investigated the preliminary phytochemical analysis of various extracts of leaves of *C. pedata* and showed the presence of carbohydrates, flavonoids, tannins and phenolic compounds and terpenes.

Qualitative analysis of Vitamins

The vitamins of the *Erythrina indica* leaves investigated and summarized in Table 3. The vitamin analysis of *Erythrina indica* leaves showed that the presence of Vitamin E while A, C and D were absent. Vitamins are organic substances that are essential in tiny amounts for growth and activity of the body. They are obtained naturally from plant and animal foods. Organic in this definition refers to the chemistry and molecules of vitamins. The word organic means that the molecules of the substance contain the element carbon. The term also means that vitamins can be destroyed and become unable to perform their functions in our bodies. Too much heat, certain kinds of light and even oxygen can destroy some vitamins. Vitamins work with other substances in the body like enzymes and minerals. Together they perform such functions as strengthening bones, healing wounds, keeping the skin healthy, building cells, and helping to resist infections. The amounts of vitamins ingested from food are measured in micrograms or milligrams (Okwu, 2004).

Histochemical analysis of leaves powder of *Erythrina indica* leaves

Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues, it is a powerful tool for localization of trace quantities of substances present in biological tissues. Histochemical techniques have been employed to characterize structure and development, and to study time course of deposition and distribution of major phyto compounds (Krishnan *et al.*, 2001). In the present study, *Erythrina indica* leaves were

treated with specific chemicals and reagents. The *Erythrina indica* leaves powder treated with diluted ammonia and H_2SO_4 gave yellow colour indicates flavonoids, treated with $FeCl_3$gave green colour indicates tannin and treated with concentrated H_2SO_4gave yellow colour indicates saponin (Table 4 and Plate 6**)**. This results further confirmed the presence of phytochemicals.

John Peter Paul, (2014) attempt was taken for histochemical and fluorescence analysis of *Turbinaria ornata* (Turner). Histochemical analyses of the plant were carried out using light microscopy and fluorescence study was analyzed by UV lamp. Results of histochemical tests showed positive reaction to phenol compounds, polyphenol and tannin in the thallus. Fine powder and different solvent extracts of *Turbinaria ornata* obtained using petroleum ether, benzene, chloroform, acetone, ethanol and aqueous were examined under visible and UV light.

Fluorescence behavior of *Erythrina indica* leaves powder

Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant. Some constituents show fluorescence in the visible range in daylight. The ultra violet light produces fluorescence in many products, which do not visibly fluorescence in daylight. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. (Tyler *et al.,* 1976).

Fluorescence analysis of entire leaves of *Erythrina indica* leaves has been carried out in daylight and under UV light. Fluorescence analysis of leaf powder of *Erythrina indica* leaves was carried out by the treatment of different chemical reagents such as $AlCl_3$, H_2SO_4, HCl, NH_3, HNO_3, CH_3OH and NaOH. The powders were observed in normal daylight and under short (245 nm) and long UV light (365 nm) and the results were presented in Table 5 and Plate 7.

In the fluorescence analysis, the plant parts or crude drugs may be examined as such or in their powdered form or in solution or as extracts. Although, in most of the cases the actual substances responsible for the fluorescence properties has not been identified, the merits of simplicity and rapidity of the process makes it a valuable analytical tool in the identification of plant samples and crude drugs (Denston, 1946). Hence, some drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation (Kokashi *et al.,* 1958).

The UV-VIS profile (Fig.1) of the *Erythrina indica* leaves extract was studied at a wavelength range of 340 to 940 nm. Three major bands were recorded at 390, 470 and 630 nm. The result confirms the occurrence of peaks at340-940 nm reveals that the absorption bands are due to the presence of flavonoids, phenol and its derivatives (Liu et al., 2006). The result of UV-VIS spectroscopic analysis confirms the presence of phenolic compounds in the extract of *Erythrina indica* leaves.

Ultraviolet/visible (UV/VIS) spectroscopy

UV-Visible spectrophotometry technique is simple, rapid, moderately specific and applicable tosmall quantities of compounds. UV-visible spectroscopy can be performed for qualitative analysis and for identification of certainclasses of compounds in both pure and biological mixtures. Preferentially, UV-visible spectroscopycan be used for quantitative analysis because aromatic molecules are powerful chromophores in theUV range. Natural compounds can be determined by using UV-visible spectroscopy. Phenoliccompounds including anthocyanins, tannins, polymer dyes, and phenols form complexes with ironthat have been detected by the ultraviolet/visible (UV-Vis) spectroscopy (Kemp, 1991).

Antimicrobial activity

Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Emergence of pathogenic microorganisms that are resistant/multi-resistant to major class of antibiotics has increased in recent years due to indiscriminate use of synthetic antimicrobial drugs. In addition, high cost and adverse side effects are commonly associated with popular synthetic antibiotics, such as hypersensitivity, allergic reactions, and immunosuppressant and are major burning global issues in treating infectious diseases (Karaman *et al*., 2003).

This situation forced scientists to search for new antimicrobial substances with plant origin. Plant extract of *Erythrina indica* leaves were screened against *Escherichia coli* species of bacteria and *Candida albicans* species of fungi were evaluated using the standard agar disc diffusion method. The disc diffusion method is used to detect the antimicrobial activity of plant extract. The solidified Nutrient agar plates were swapped with the test organism and the samples were impregnated. After the incubation the zone was measured. The antimicrobial activity of plant extracts was detected by the indication of zone around the disc. The *in vitro* antimicrobial activity of the *Erythrina indica* leaves extract against these bacteria and fungi were qualitatively

assessed by the presence of inhibition zones represented in the photographic plate 8. The inhibitory activities in culture media of the microbes reported in Table 6 were comparable with standard antimicrobiotic viz. chloramphenicol and fluconazole.

Table.1: Qualitative analysis of Phytochemicals in *Erythrina indica* leaves

S.No	Test analysis	Methanol extract	Aqueous extract
1	Tannin	+	+
2	Phlobatannin	-	-
3	Saponin	+	+
4	Flavanoids	+	++
5	Steroids	+	+
6	Terpenoids	+	+
7	Triterpnoids	+	++
8	Alkaloid	-	++
9	Carbohydrate	+	+
10	Protein	+	+
11	Anthroquinone	+	+
12	Polyphenol	+	+
13	Glycoside	+	+

(-) Indicates Absence; (+) Indicates Presence

Table.2: Quantitative phytochemical analysis of *Erythrina indica* leaves extract

S.No	Secondary Metabolites	Result (mg/gm)
1	Phenol	77.60±5.43
2	Flavonoids	110.00±7.70
3	Terpenoids	9.00±0.63

Values are expressed as mean ± SD for triplicates

Table.3: Qualitative analysis of vitamins in *Erythrina indica* leaves

S.ON	Vitamins	Result
1	Vitamins A	-
2	Vitamins C	-
3	Vitamins D	-
4	Vitamins E	+

(+) Presence, (-) Absence

Table.4: Histochemical analysis of leaves powder of *Erythrina indica* leaves

S.No	Charecterisation	Observation	Result
1	Tannin	Green	+
2	Flavonoids	Yellow	+
3	Saponin	Yellow	++
4	Steroids	Green	++
5	Terpenoids	Orange	+
6	Alkaloid	Reddish brown	+
7	Glycoside	Brown	++
8	Polyphenol	Blue green	+

Note: (+) Presence; (++) present with high intensity of the colour

Table.5: Fluorescence behavior of *Erythrina indica* leaves powder

S.No	Test	Visible Light	Short UV	Long UV
1	Plant powder	Green	Green	Green
2	Plant powder treated with water	Green	Green	Black
3	Plant powder treated with Hexane	Green	Green	Black
4	Plant powder treated with Chloroform	Green	Green	Black
5	Plant powder treated with Methanol	Green	Dark green	Black
6	Plant powder treated with Acetone	Green	Green	Black
7	Plant powder treated with 1N NaOH (water)	Green	Green	Black
8	Plant powder treated with 1N HCl	Brownish green	Black	Violet
9	Plant powder treated with sulphuric acid with an equal amount of water	Dark green	Dark black	Green
10	Plant powder treated with Nitric acid dilute with an equal amount of water	Yellow	Light green	Violet

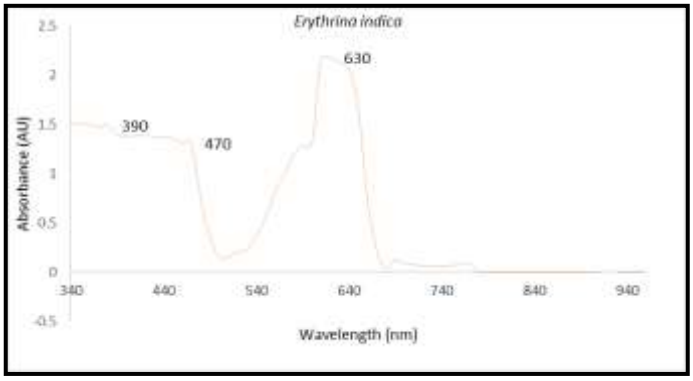

Fig.1: UV-Visible spectrum analysis of *Erythrina indica* leaves

Table.6: Antimicrobial activities of *Erythrina indica* leaves in Methanol extract

Microbial Organism	50µl	100 µl	150 µl	Standard
Escherichia coli (mm)	12.50±0.87	13.25±0.92	14.25±0.99	16.25±1.13
Candida albicans (mm)	7.00±0.49	14.75±1.03	14.25±0.99	14.50±1.05

Values were expressed as Mean ± SD. Bacterial standard – Chloramphenicol
Fungal standard - Fluconazole

BACTERIA **FUNGI**

Plate.8: Antimicrobial activities of *Erythrina indica* leaves

CONCLUSION

Overall, it can be concluded from the present study that *Erythrina indica* leaves contains rich source of phytochemicals. This study is the first scientific report that provides convincing phytochemicals evidence for the relevance of *Erythrina indica* leaves thus providing scientific validity to its traditional consumption by the local population of south India.

REFERENCES

Awoyinka, O., Balogun,IO., Ogunnowo,AA., (2007). Phytochemical screening and *invitro* bioactivity Of Cnidoscolus aconitifolius (Euphorbiaceae). *J Med Plant* ,V.1(3): p.63-65.

Bohm BA and Kocipai-Abyazan R. (1994) Flavonoids and condensed tannins from leaves of *Hawaiian vaccinium* vaticulatum and V. calycinium.*Pacific Sci*. 48: 458-463.

Denston TC. (1946) "A textbook of Pharmacognosy", Sir Isaac Pitman & Sons, Ltd, London, 46-51.

Edeoga HO, Okwu DE and Mbaebie BO. (2005) Phytochemical constituents of some Nigerian medicinalplants. African Journal of Biotechnology, 4 (7): 685-688.

Ferguson NM. A Text Book of Pharmacognosy. New Delhi: Mac Milan Company; 1956. p. 191.

.Harborne JB (1973). *Phytochemical methods*, London. Chapman and Hall, Ltd. pp. 49-188.

Karaman L, Sahin F, Gulluce M, Ogutcu H, Sngul M, Adiguzel A. (2003) Antimicrobial activity of aqueous and methanol extracts of Juniperus oxycedrus L. J Ethnopharmacol.; 85: 231-235.

Kotnis MS, Patel P, Menon SN and Sane RT. (2004) Reneprotective effect of Hemisdesmus indicus, a herbal drug used in gentamicin-induced renal toxicity. Nephrology (carlton), 3:142-152.

Kumar M, Mondal P, Borah S and Mahato K. (2013) Physico- chemical evaluation, preliminary phytochemical investigation, fluorescence and TLC analysis of leaves of the plant Lasia spinosa (Lour) Thwaites. Int J Pharm Pharm Sci, 5 (2):306-310.

NCCLS-National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disc susceptibility tests. PA: NCCLS Publications; 1993, p. M2-A5.

Patel DK, Kumar R, Prasad SK and Hemalatha S. (2011) Pedalium murex Linn (Pedaliaceae) fruits: A comparative antioxidant activity of its different fractions. *Asian Pac J Trop Biomed* 1: 395-400.

Pearson Thomas A .Cardiovascular Disease in Developing Countries: Myths, Realities, and Opportunities. Cardiovascular Drugs and Therapy 1999;13:95–104

Rao VV, Gurundhra S, Dhar RL, Subramanyam K (2001) Assessment of

Reena Ganesan, Kamalanathan Desingu, Ragavendran Chinnasamy and Natarajan Devarajan.,(2013). Screening of Antibacterial and Phytochemical analysis of leaf and leaf derived Callus extracts of *Sebastiania chamaelea* (L.) Muell. Arg. Indo American Journal of Pharmaceutical Research, 2231-6876.

Sofowara A, 1982. Medicinal plants and traditional medicines in Africa, John Wiley and Sons, New York, pp 256-259.

Trease GE and Evans WC. (1989) Phenols and Phenolic glycosides. In:Textbook of Pharmacognosy. (12th ed.). Balliese, Tindall and Co Publishers, London pp. 343-383.

Van-Burden TP and Robinson WC.(1981) Formation of complexes between protein and Tannin acid.*J. Agric. Food Chem*, 1: 77.

Source of support: Nil;
Conflict of interest: None declared