IJASRM

International Journal of Advanced Scientific Research and Management, Volume 4 Issue 3, Mar 2019

www.ijasrm.com

ISSN 2455-6378

Phytochemical screening and Invitro antioxidant activity of Lantana camara

Sasi Premila J.M[1], Kala Vetha Kumari [S2] and Sreeja N[3]

[1] Associate Professor,Department of Biotechnology, Annai Velankanni College, Tholayavattam, Tamil Nadu, India

[2] Assistant Professor,Department of Biotechnology, Annai Velankanni College, Tholayavattam, Tamil Nadu, India.

[3] Department of Biotechnology, Annai Velankanni College, Tholayavattam, Tamil Nadu, India

Abstract

The present study was carried out to find out the phytochemicals, antimicrobial activity and to determine the antioxidant activity of different extract of Lantana camara. The antioxidant activity of the plant extracts were determined by DPPH radical scavenging assay. Phytochemical analysis revealed the presence of Tannin, saponin, Terpenoids, flavonoids on ethanol and methanol extracts. The antimicrobial activity of ethanol, methanol, acetone and petroleum ether extracts of Lantana camara was evaluated using disc diffusion method. The results of the present study clearly indicated that ethanol extract showed highest reducing power activity in Lantana camara at concentration of 100 mg/ml of extract. The obtained results suggested that the ethanol extract of the leaves of Lantana camara possess antimicrobial and antioxidant.

Key words: - *Bioactive molecules, pharmaceutical products, antioxidant activity*.

1. Introduction

Natural products perform various functions, and many of them have interesting and useful biological activities [1]. Phytochemicals or secondary metabolites are chemical compounds formed during the plants normal metabolic processes and plants use them to protect themselves [2]. About 121 drugs prescribed in USA today come from natural sources, 90 of which come either directly or indirectly from plant sources. Forty-seven percent of the anticancer drugs in the market come from natural products or natural product mimics [3]. Antimicrobial activities have been detected in chemicals extracted from root, stem, leaves, flowers and seeds of diverse species of plants. In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents [4]. L. camara is a well-known medicinal plant in traditional medicinal system and recent scientific studies have emphasized the possible use of L. camara in modern medicine. Its resilient nature makes the plant invasive and widely distributed in the pantropic. Lantanacamara leaves can display antimicrobial, fungicidal and insecticidal properties. L. camara has also been used in traditional herbal medicines for treating a variety of ailments, including cancer, skin itches, leprosey, rabies, chicken pox, measles, asthma and ulcers.

2. Materials and methods

2.1 Collection of plant materials

The plant specimens, Lantana camara for the proposed study was collected from Poottety, Kanyakumari District and these specimens were identified on the basis of morphological characterizes and comparison with voucher specimens recorded in the Central Herbarium of Botanical Survey of India.

2.2 Preparation of samples

The healthy leaves of Lantana camara were air dried under shade. The leaves were then powered, sieved and stored in an air tight container for examination.

2.3 Preparation of solvent extracts

About 50g of powdered material was successively extracted with 250ml of solvents such as ethanol, acetone, petroleum ether and methanol. They were placed in shaker for 3 days and the extract was collected from the conical flask by filtration.

Then the plant extract was kept in a water bath at 60°C to evaporate the solvent from the solution.

2.4 Phytochemical screening

The preliminary qualitative analysis of the plant extracts were performed to screen for the presence of bio active compounds in the Lantana camara leaves (Evans W C, 1989).

2.4.1. Test for alkaloids
To 1ml of the filtrate, a drop of Mayer's reagent was added along the side of the test tube. The test solution was observed for the presence of yellowish or white precipitate.

2.4.2. Test for tannin
To 1ml of the sample, 1ml of ferric chloride solution was added. The test solution was then observed for the presence of black or green precipitate.

2.4.3. Test for saponin
To 2ml of the sample, 5ml of distilled water was added shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously and observed for the formation of emulsion.

2.4.4. Test for carbohydrate
To 1ml of the extract, added 2-3 drops of 1% alcoholic alpha-naphthol and 2ml concentrated sulphuric acid. This was added along the sides of the test tube (violet ring at the junction of two layers).

2.4.5. Test for terpenoids
To 5ml of the sample, 2ml of chloroform and concentrated sulphuric acid was carefully added to form layer. It was observed for the formation of reddish brown coloration at the interphase.

2.4.6. Test for flavonoids
To 5ml of the dilute ammonia solution a portion of the aqueous filtrate of each sample followed by addition of concentrated sulphuric acid. It was observed for the formation of yellow coloration.

2.4.7. Test for steroids
To 1ml of the aqueous extract, 10ml 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 indicates the presence of steroids.

2.4.8. Test for amino acids
To 1ml of the extract, added few drops of ninhydrin reagent. Appearance of purple color shows the presence of amino acids.

2.4.9. Test for phenols
To 1ml of each extract dissolved in alcohol or water, separately added few ml of neutral ferric chloride solution. Any change in color indicates the presence of phenolic compounds.

2.4.10. Test for protein
To 1ml of diluted extract, 1ml of 5% $CuSO_4$ and 1% of NaOH solution was added. Deep blue color confirmes the presence of proteins.

2.5 Antibacterial assay

Antibacterial assay of plant leaf extracts were determined using Kirby Bauer disc diffusion method. The test microorganisms were spreaded on the petri plates and sterile disc (6mm) impregnated with plant leaf extracts are placed and incubated at 37°C for 24 hours. Check for antimicrobial activity, by looking for the clear area called zone of inhibition was measured in millimeters and compared with antibiotic disc Kanamycin.

2.6 Phenolic content assay

Crude methanolic extract of 100µl and 100-500µl of Gallic acid as working standard were made up to 500µl and then mixed with 1.5ml of Folin- Ciocalteu reagent. 1.5ml of Sodium carbonate solution was added to the above mixture. After 90min incubation at room temperature, absorbance was measured at 725nm.

3. Results & Discussion

The quantitative screening of Lantana camara leaves revealed the presence of phytochemical such as alkaloids, tannin, saponin, amino acid and phenol in acetone whereas carbohydrates, flavonoids, terpenoids, steroids and protein are absent. The phytochemical test revealed the presence of tannin, saponin, terpenoids, flavonoids and steroids in methanol, whereas alkaloids, carbohydrates, amino acid, phenol and protein are absent (table 4.1).Ethanol extracts revealed the presence of tannin, saponin, terpenoids and flavonoids whereas phytochemical compounds were absent in petroleum ether extract.

Plants produce a number of substances and most are secondary metabolites which act as defense against predators, responsible for typical odors and characteristic pigmented nature of plants. Most of the phytochemicals are extensively used as medicinal compounds for treatment of various ailments all over the world [5]. On screening plant extracts for antimicrobial activity, it has been shown that higher plants represent a promising source of new antimicrobial agents [6].

Phytochemical analysis of Lantana camara revealed the presence of saponin and terpenoids. [7] reported that secondary metabolites such as terpenoids, tannin, saponin, could be held partially responsible for some of the biological activity. The present results are compared with [8,9] The presence of tannins in the

extract indicated the possible use of the plants in ethanobiological medicine. The tannins have the ability to reacts with protein, forming stable water insoluble components and therefore, may have a profound effect on bacteria since bacterial cell wall are made up of proteins [10].

Antibacterial activities of the extracts of Lantana camara were tested against ten pathogenic bacteria were compared with the standard antibiotic ampicillin by measuring the zone of inhibition diameter and expressed in millimeter (mm).

The leaf ethanol extract of Lantana camara showed high activity in Proteus vulgaris (8mm), (4.5mm in acetone), (3.2mm in methanol). Among the bacterial species most of the test organisms showed activity by zone of inhibition, against Staphylococcus aureus (5.2mm in ethanol), (0.8mm in acetone and no activity in other extracts). E.coli (7mm in ethanol), (2mm in acetone and no activity of methanol and petroleum ether). Bacillus sps (6.1mm in ethanol), (4mm in methanol),(3.2mm in petroleum ether and no activity of acetone). Aeromonasesps (4.5mm in ethanol), (2.1mm in petroleum ether), (0.9mm in acetone and methanol have no activity). Among the extracts against Lactobacillus sps (0.2mm in ethanol), (1.3mm in methanol), (0.6mm in acetone), (1.5mm in petroleum ether).Streptococcus mutant (1.9mm in ethanol), (1.5mm in methanol), (2mm in acetone and no activity in petroleum ether).The activity of various extracts were compared to those of standard antibacterial agent ampicillin (figure 4.1).

Lantana camara leaf extract off our solvents showed antibacterial activity against gram positive and gram negative bacteria. Among the four solvents ethanol extract of Lantana camara showed highest zone of inhibition against both gram positive and gram negative bacteria. The most susceptible bacteria is E.coli and Proteus vulgaris, followed by Bacillus sps. It shows that ethanolic extracts contain active compounds which are either in the form of protein or in the form of any other organic compounds. Because only the ethanolic extracts at showed the highest antibacterial property rather than other extracts. [11] also reported that ethanolic extract showed of significant antibacterial activity. The extract nature and mode of action of the active constituents is quite obscure at this stage. Further work may however reveal whether these components act as intracellular bacterial enzyme Inhibitor or impair the cell wall synthesizing system of the cell, or any other biological reaction impairment which causes cessation and or inhibition of growth of bacterial cells.

Scavenging activity has been widely used to evaluate the antioxidant activity of plant extracts of Lantana camara. The presence of antioxidant in the sample extracts react with DPPH, which is a stable free radical and convert into 1,1-diphenyl -2-(2,4,6-trinitrophenyl hydrazine). The degree of discoloration indicates the scavenging potentials of the antioxidant compounds which can be detected spectrophotometrically at 517nm. The extracts are able to reduce a stable radical DPPH to the yellow colored of DPPH. The scavenging effect of ethanol extracts with the DPPH radical is (20%) at 20µg/ml (table 4.2).

DPPH is nitrogen centered stable free radical having maximum absorption at 517nm in alcoholic solution. It becomes a stable diamagnetic molecule on accepting an electron or hydrogen atom. In the presence of an extract capable of donating a hydrogen atom, the free radical nature of DPPH is to stand the purple color changes to yellow (diphenylpicrylhydrazine). DPPH radical is one of the most widely used strategies to evaluate the antioxidant activity of herbal extracts. This method is simple, rapid and measures the capacity of herbal extract to bleach the DPPH radical. The method is sensitive and requires small amount of samples [12-17]. In the present study, we monitored the decrease in DPPH absorption in the presence of varying concentrations of leaf extracts at 517nm.

The content of total phenolic in the leaf extract of Lantana camara was estimated by FCR (Folin-Ciocalteu reagent) method. Total phenolic content, as estimated in terms of mg/10g fresh weight, was high in ethanol (58) followed by acetone (42), methanol (37) and petroleum ether (31)(table 4.3)
A number of studies have focused on the biological activities of the phenolic compounds, which are the potential antioxidants and free radical- scavengers. In the present study ethanol extract of Lantana camara, contained the maximum amount of phenolics. Hence this may be a good source for phenolic compounds. Many studies have shown that natural antioxidant since plants are closely related to their bio functionalities such as the reduction of chronic diseases and inhibition of pathogenic bacterial growth, which are often associated with the termination of free radical propagation in biological systems. The phenolic compounds are also thought to contribute directly to the antioxidant activity. [18, 19] suggested a correlation between phenolic content and antioxidant activity.

Reducing power is novel anti-oxidation defense mechanisms. The two mechanisms that are available to effect this property are electron transfer and hydrogen atom transfer the reducing power of four extracts was assessed based on their ability to reduce Fe (111) to Fe (11) and the results are presented as ascorbic acid equivalent. The results had a higher reducing power. Among the four extracts ethanol (85

mg) had higher reducing power Lantana camara (table4.4).

The assay has been widely used by several researchers to evaluate antioxidant activity of compounds [20-26]. In this assay, the presence of reductants (antioxidants) in the samples would result in the reduction of Fe+3 to Fe+2 by donating an electron [27]. The reducing nature of a compound may serve as a significant indicator of its potent antioxidant activity. As in case of DPPH assay, the extracts containing high phenolic contents displayed greater reducing power. It is evident from the study that the extracts possess reducing power and therefore, could serve as electron donors, terminating the radical chain reactions [27].

4. Tables and Figures

Table 4.1: Phytochemical test results of extract of Lantana camara leaves

Phytochemicals	Ethanol	Acetone	Methanol	Petroleum ether
Alkaloid	-	-	-	+
Tannin	+	+	+	-
Saponin	+	+	+	-
Carbohydrate	-	-	-	-
Terpenoids	+	-	+	+
Flavanoids	+	-	+	-
Phenol	-	+	-	+
Aminoacids	-	+	-	-
Protein	+	-	-	-
Steroids	-	-	+	+

Figure 4.1: Antibacterial activity of extracts of Lantana camara

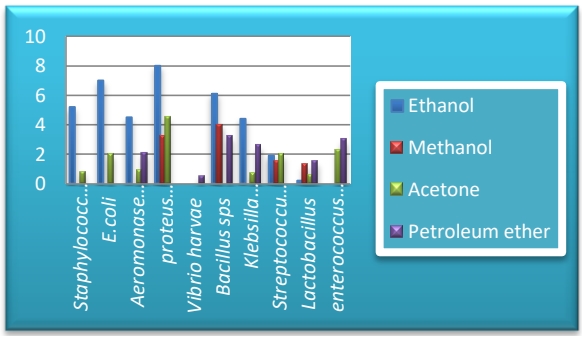

Table 4.2. DPPH Radical Scavenging Activity of Lantana camara

CONCENTRATION (µg/ml)	ETHANOL (%)	STANDARD
20	20	14
40	21	28
60	10	42
80	13	57
100	13	71
120	13	85
140	14	99
160	13	114
180	14	128

Table 4.3. TOTAL PHENOLIC ACTIVITY OF Lantana camara

S.NO	**PLANT EXTRACTS**	***mg/10g of fresh wt. | Lantana camara***
1	Ethanol	58
2	Methanol	37
3	Acetone	42
4	Petroleum ether	31

Table 4.3. REDUCING POWER ASSAY OF Lantana camara

S.NO	**EXTRACTS**	**mg AAE/100g of fresh weight**
1	Ethanol	85
2	Methanol	80
3	Acetone	70
4	Petroleum ether	68

5. Conclusion

Demand to herbal drugs is increasing day by day. Plants contain number of chemical moieties with varied pharmacological activities. Many potent and efficous medicinal principles used for treating dreadful diseases have been isolated from plant kingdom. So it is very clear that the study of the medicinal plant is important to meet the requirements in effective therapy. Hence the work is mainly concentrated to separate bioactive compounds and the use traditional medicine for diseases. From the present investigation it is concluded that species have high ethanobotanical compounds so it can be treated for various infection caused by human pathogenic microorganisms. Lantana camara may be considered as a valuable plant in both ayurvedic and modern drug development areas of its versatile medicinal uses.

References

[1] Galal M, Bashir A K, Salih A M, and Adam S E I, Activity of water extracts of Albizia anthel mintica and A. lebbek backs against exprimental Hymenolepis diminuta infection in rats. J. Ethnopharmacol., 31: 333-337 , (1991).
[2] Alison AW, George W J F, Naoki A, Russell J M, Robert JN, Polyhydroxylated alkaloids - natural occurrence and therapeutic applications. Phytochemistry; 56: 265-295, (2001).
[3] Ravalli Remella, Nageswara Rao K V V, Bhimji Ambedkaru K, Extraction and Evaluation of Anti-oxidant Activity of Hibiscus Cannabis. L, Research and Reviews: Journal of Pharmacognosy and Phytochemistry. JPRPC | Volume 3 | Issue 1.E-ISSN: 2321-6182 P-ISSN: 2347-2332, (2015).
[4] Balandrin M F, Kjocke A J, Wurtele E, Natural plant chemicals: sources of industrial and mechanical materials. Science 228: 1154-1160, (1985).
[5] Cowan M M, Plant products as antimicrobial agents. Clinical Microbiology Reviews; 12(4): 564-582, (1999).
[6] Ojala T, Remes S, Haansuu P, Vuorela H, Hiltunen R, Haahtela K, Vuorela P. Antimicrobial activity of some coumarin containing herbal plants growing in Finland. Journal of Ethnopharmacology.; 73: 299– 305, (2000).
[7] Barre F S Antibacterial activity of Lantana camara Linn and Lantana montevidensis Brig extracts from Cariri-Ceará, Brazil. Journal of Young Pharmacists.2 (1): 42-44,(1999).
[8] Samy PR, Ignacimuthus S, Raja DP.: Preliminary screening of ethnomedicinal plants from India. J.Ethanopharmacol 66(2): 235-240, (1999).
[9] Hinneburg I, Dorman HJD, Hiltunen R, Antioxidant activities of extracts from selected culinary herbs and spices. Food Chemistry; 97: 122-129, (2006).
[10] TreaseGEandEvansWCPharmacognosy,13thed ition, Bailere Traiadal, London, P P: 378:386-480,(1989).
[11] Gupta Raj Narayan, Viswas Kartik, Pathak Manoj, Parihar Surendra Singh, Gupta, Antibacterial activities of ethanolic extracts of plants used in folk medicine. IJRAP1(2)529-535, (2010).
[12] Kulisic T, Radonic A, Katalinic V, Milos M, Use of different methods for testing antioxidative activity of oregano essential oil. Food Chem 85: 633–640, (2004).
[13] Kaviarasan S, NaikG H, Gangabhagirathi R, Anuradha C V, Priyadarshini KI, Invitro studies on antiradical and antioxidant activities of fenugreek (Trigonella foenum graecum) seeds. Food Chemistry. 103: 31-37, (2007).
[14] LetelierME,Molina-BerriosA,Cortes-TroncosoJ,Jara-SandovalJ,HolstM,PalmaK, MontoyaM, MirandaD and Gonzalez-LiraV, DPPH and oxygen free radicals as pro-oxidant of biomolecules. Toxicology In vitro; 22: 279–286, (2008).
[15] KekudaTRP, VinayakaKS, SwathiD, SuchithaY, VenugopalTM and MallikarjunN. Mineral composition, total phenol content and antioxidant activity of a Macrolichen Everniastrum cirrhatum (Fr.)Hale (Parmeliaceae). E- Journal of Chemistry. 8(4): 1886-1894, (2011).
[16] Junaid S, Rakesh KN, DileepN, Poornima G, Kekuda TRP and MukundaS, Total phenolic content and antioxidant activity of seed extract of Lagerstroemia speciosa L. Chemical Science Transactions; 2(1): 75-80, (2013).
[17] Pavithra GM, Siddiqua S,NaikAS,Kekuda PTR,Vinayaka KS, Antioxidant and antimicrobial activity of flowers of Wendlandiathyrsoidea, Oleadioica, Lagerstroemia species and Bombax malabaricum. Journal of Applied Pharmaceutical Science. 3(6) , (2013).
[18] Yen GC, Duh PD, Tsai CL, Relationship between antioxidant activity and maturity of peanut hulls. J Agric Food Chem 41:67-70, (1993).
[19] Duh PD, TuYY, YenGC, Antioxidant activity of water extract of HarngJyur (Chrysanthem ummorifolium Ramat). Leb-Wis Technol 32:269-277. 35,(1999).
[20] Yuan YV, Bone DE, Carrington MF, Antioxidant activity of dulse (Palmaria palmata) extract evaluated in vitro. Food Chemistr; 91: 485-494,(2005)
[21] Hinneburg I, Dorman HJD, Hiltunen R, Antioxidant activities of extracts from selected culinary herbs and spices. Food Chemistry; 97: 122-129, (2006).
[22] Kim S, Jeong S, Park W, Nam KC, Ahn DU, Lee S, Effect of heating conditions on grape seeds on the antioxidant activity of grape seed extracts. Food Chemistry. 97: 472-479, (2006).
[23] Barros L, Falcao S, Baptista P, Freire C, Vilas-Boas M, Ferreira I C F R, Antioxidant activity of Agaricus sp. mushrooms by chemical, biochemical and electrochemical assays. Food Chemistry; 111: 61- 66, (2008).
[24] Gulcin I, Topal F, Sarikaya SBO, Bursal E, Bilsel G, Goren AC, Polyphenol contents and antioxidant properties of Medlar (Mespilus germanica L.). Records of Natural Products; 5(3): 158-175,(2011).
[25] Rekha C, Poornima G, Manasa M, Abhipsa V, Devi P J, Kumar V H T, Kekuda P T R, Ascorbic Acid, total phenol content and antioxidant activity of fresh juices of four ripe

and unripe Citrus fruits. Chemical Science Transactions. 1(2): 303-310, (2012).

[26] Junaid S, Rakesh K N, Dileep N, Poornima G, Kekuda T R P, Mukunda S, Total phenolic content and antioxidant activity of seed extract of Lagerstroemia speciosa L. Chemical Science Transactions; 2(1): 75-80, (2013).

[27] Chung Y, Chien C, Teng K, Chou S, Antioxidative and mutagenic properties of Zanthoxylum ailanthoides Sieb & zucc. Food Chemistry; 97: 418-425, (2006).