Studies on antioxidant activities of *Lantana camara* extracts

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Abstract : The present investigation deals with the extraction, phytochemical constituent analysis and antioxidant activity analysis of *Lantana camara*. Solvents of varied polarity were employed for the extraction and it was observed that the yields of extraction depend on nature of solvent. Ethanol and methanol were the best possible solvents for having higher yields of the extract compared to other solvents chosen of varied polarities. Polyphenol content determination indicates that the ethanol and methanol extracts were found to have highest percent of polyphenols than other extracts. The Flavonoid estimation of the extracts indicate that methanol extract possess highest amount of flavonoids among all the extracts of different solvents. The DPPH inhibition activity analysis indicates that water extract evidenced to have potential antioxidant activity among all extracts of different solvents. This is probably due to the considerably high flavonoid content compared to other extracts. It is also clear that *Lantana camara* seems to have highly soluble compounds in ethanol and methanol; hence the yield is also high. Water extract emerges out as the best possible extract in terms of antioxidant activity. But methanol, ethyl acetate and ethanol extracts are shown to high higher yields. This investigation points out that the *Lanatana camara* contains significant amounts of antioxidant compounds such as polyphenols and flavonoids. Consequently various extracts showed potential antioxidant activity through DPPH free radical scavenging activity studies. Hence it is worth to consider *Lantana camara* for further exploration in utilizing it as rich antioxidant.

***IndexTerms - Lanatana camara*, antioxidant, polyphenols, flavonoids.**

I. INTRODUCTION

Lanatana camara (*L. camara*) is a rugged evergreen shrub growing to 1.8 m high. Stems are square in profile, with small prickles. The leaves are arranged in opposite pairs, they are broadly oval in shape, rough short hairs, with finely toothed edges. Flowers are a mixture of cream, pink or orange numerous small rounded heads, often in two colours, yellow and red. Fruits are fleshly berries in cluster, green ripening to black. *Lantana camara*, also known as Spanish Flag or West Indian Lantana, is a species of flowering plant in the verbena family, Verbenaceae, that is native to the American tropics. It has been introduced into other parts of the world as an ornamental plant and is considered an invasive species in many tropical and sub-tropical area. *L. camara* is sometimes known as "Red (Yellow, Wild) Sage", despite its classification in a separate family to sage (Lamiaceae), and a different order to sagebrush (Asterales). The native range of *Lantana camara* includes Mexico, Central America, the Greater Antilles, The Bahamas, Colombia, and Venezuela. It is believed to be indigenous to the Lower Rio Grande Valley of Texas in the United States. It has become naturalized in tropical and warm regions worldwide. In the Kenyan highlands it grows in many areas that receive even minimal amounts of rainfall. It can be seen in the wild and along footpaths, deserted fields, and farms. West Indian Lantana has been naturalized in the United States, particularly in the Atlantic coastal plains, from Florida to Georgia, where the climate is close to its native climate, with high heat and humidity.

Flowers of *L. camara* with white crab spider (*Misumenoides formosipes*, lower right) in wait for prey. *L. camara* is an invasive species and has covered large areas in India, Australia and much of Africa. It colonizes new areas when its seeds are dispersed by birds. Once it reaches an area, *L. camara* spreads quickly. It coppices so well, that efforts to eradicate it have completely failed. It is resistant to fire, and quickly grows in and colonizes burnt areas. It has become a serious obstacle to the natural regeneration of important native species including the Shala Tree (*Shorea robusta*) in Southeast Asia, as well as plants in 22 other countries. In greenhouses, *L. camara* is notorious for attracting whitefly. In India they bear fruit all year round and this appears to have an impact on bird communities. While considered a pest in Australia, it shelters several native marsupial species from predators, and offers a habitat for the vulnerable *Exoneura* native bee, which nests in the hollow stems of the plant. *L. camara* has been listed as a Category One "Invasive Toxic Species" in Florida by the Florida Exotic Pest Plant Council, and has become a problem in Texas and Hawaii.

Belonging to the plant family Verbanaceae, *L. camara* bears various groups of chemical components Mono- and sesquiterpenes such as curcumenes, bisabolene and safrole is present in oils of leaves and flowers. Triterpenes such as lantadenes A, B, C and D, lantanolic and lantic acid in leaves and stems and oleanolic in roots was found. Five euphane triterpene lactones from the methanolic extract of *L. camara* were found and said to be the potent inhibitors of human thrombin. Iridoid glycosides such as theveside present in stems and leaves as sodium salt and geniposide, 8-epiloganin, lamiridoside and shanzide methyl ester. Flavonoids such as multiple methoxylated derivatives of quercetin (3-methoxy-, 3, 7-dimethoxy-, 3, 7,4`-trimethoxy-) and camaroside from leaves and Hispudoline from the stems and from dried leaves verbascoside, an inhibitor of protein kinase C and umuhengerin (polymethoxylated flavone) with antimicrobial property was found. Essential oil from the leaves contain an oil rich of sesquiterpenes and aerial parts contains ß-caryophyllene, geranyl acetate, terpinyl acetate, bornylacetate and D-limonene. The

essential oil showed antibacterial and antifungal activity. Miscellaneous compounds found in the stem are steroids like ß-sitosterol, campesterol, stigmasterol, ß-sitosterolglucoside and five oligosaccharides like ajugose, stachyose, verbascose, verbascotetraose and lantanose. Form the roots verbascoside, isoverbascoside, martynoside, D-rhamnosylverbascoside and some other phenylethanoides and quinone diodantunezone (in hexane extract).
Thus the plant is said to possess principle constituents like alkaloids terpenoids phenolics, flavonoids and steroids (1). Several classes of compounds such as mono- and sesquiterpenes, Triterpenes, Iridoid glycosides, furanonaphthoquinones, flavonoids, and phenylethanoides glycosides have been reported to be present in this genus. The stem is said to contain three pentacyclic triterpenoids such as oleanolic acid , betulonic acid, and ursolic acid, oleanonic acid , lantadene , lantadene A, lantadene B, lantanilic acid, betulonic acid, betulinic acid pomolic acid and flavonoids like hispudoline and camaroside (2) were also verified by comparing their spectral data with the literature value.

Kidney is major organ systems physiologically involved in the metabolism and excretion of various xenobiotics, environmental pollutants etc. Consequently they are exposed to oxidative stress and free radicals. This results in the tissue necrosis and damage of this organ system. Therefore several attempts have been made to protect this organ from the free radical challenges. As we gone through various studies on treatment of kidney disorder, herbal plants play a unique role in medicine. There are no synthetic drugs which relieve overall insufficiency of kidney. But indigenous plants possess tissue rejuvenator property which is anyway unavoidable. Indians who are brought upon Indian food, soul and climate with Indian habits of life and environment, Indian drugs naturally suit better and safer than European constitution built upon their peculiar food, climate, habits and manner of life. This may perhaps be the reason why in numerous cases, where synthetic medicine fails, Indigenous system of medication succeed. A large number of medicinal plants, natural products dietary components have been evaluated as potential nephroprotective agents (3). Free radicals form when oxygen is metabolized or formed in the body and are chemical species that possess an unpaired electron in the outer (valence) shell of the molecule. This is the reason why free radicals are highly reactive and can react with proteins, lipids, carbohydrates and DNA. These free radicals attack the nearest stable molecule, stealing its electron. When the attacked molecule loses its electron, it becomes a free radical itself, beginning a chain reaction, finally resulting in the disruption of a living cell. Free radicals may be either oxygen derived (ROS, reactive oxygen species) or nitrogen derived (RNS reactive nitrogen species). The oxygen derived molecules are O_2 [superoxide], HO [hydroxyl], HO_2 [hydroperoxyl], ROO [peroxyl], RO [alkoxyl] as free radical and H_2O_2 [hydrogen peroxide], HOCl [hydrogen peroxide], HOCl [hypochlorus acid], O_3 [ozone] and O_2 [singlet oxygen] as non-radical. Nitrogen derived oxidant species are mainly NO [nitric oxide] ONOO [peroxy nitrate], NO_2 [nitrogen dioxide] and N_2O_3 [dinitrogen trioxide] (4).

The lipid peroxidation initiates cascade of biochemical reactions leading to cellular necrosis. However, there are certain inbuilt antioxidant systems like tissue GSH, superoxide dismutase, catalase etc. to scavenge the free radicals and protect the organs. Free radical injury and oxidative stress has been implicates in many renal diseases like acute renal failure, IgA nephropathy, anemia of chronic renal failure and ischemic kidney.

Renal system is highly prone to attack by generation of excessive concentration of free radicals. Many drugs, chemicals, pollutants, may cause toxicity to this organ through mechanism mentioned above. Many antioxidants have been used to protect the organs from the free radical challenges and most of the antioxidants are of natural origin. Antioxidant nature of the phenolics has been well documented in a number of plants. The key role of phenolics compounds as scavengers of free radicals is emphasized in several previously published reports. Various classes of phytoconstituents are well established to be antioxidant in leaves of *Lantana camara*, such as phenolics, flavonoids, tannins, and proanthocyanidins. Several researches are attempting to explore the possibility of using herbs containing antioxidant principles as organ protective agents, where studies reported that flavonoids from *Drynaria fortunei* plant have prevented nephrotoxicity, improved kidney function and promotes kidney primary epithelial tubular cell regeneration (4). Keeping in tone with this researcher in our laboratory under take filed survey's and contact native practitioners so as to identify and assess the locally available herbs for their usefulness as organ protective agents.

Lantana camara linn, belongs to family V*erbanaceae* and it is a reservoir of several important bioactive molecules. It has been listed as one of the important medicinal plants of the world. The plant is said to possess principle constituents like alkaloids, terpenoids, phenolics, flavonoids and steroids. Several classes of compounds such as mono- and sesquiterpenes, Triterpenes, Iridoid glycosides, furanonaphthoquinones, flavonoids, and phenylethanoides glycosides have been reported to be present in this genus. The stem is said to contain three pentacyclic triterpenoids such as oleanolic acid , betulonic acid, and ursolic acid, oleanonic acid (5), lantadene , lantadene A, lantadeneB, lantanilic acid, betulonic acid , betulinic acid pomolic acid and flavonoids like hispudoline,and camaroside were also verified by comparing their spectral data with the literature value. For many years, natural products from *Lantana* have been used in the prevention and cure of many serious diseases, including cancers, all over the world (6).

But traditionally the plant is used for treating fever, influenza, stomach ache (leaves and flower) , cold, rheumatism, asthma and high blood pressure (whole plant), sores, chicken pox, measles (leaves), stomach ache (powdered root in milk), cough (decoction of leaves), tetanus, rheumatism, malaria and ataxia of abdominal viscera (whole plant), antiseptic for wounds leaf oil (7). Further literature reveals that *Lantana camara* Linn has been reported for fistula, pustules, tumors and rheumatism(fruits), antilymphocytic, immunosuppressive, hepatoprotective, thrombin inhibitory, termiticidal, antimotility, antifilarial, *in-vitro* cytotoxic and antimicrobial activity (different parts of the plant). The leaf extract has been said to possess the wound healing and antidiabetic properties (8). The pharmacological significance was noted due to the presence of various bioactive compounds such

as lantadenes in all *L. camara*. In addition, other secondary metabolites such as alkaloids, terpenoids, and phenolics could be held partially responsible for some of these biological activities.

Flavonoids are known to possess antioxidant activity (9,10). Likely, proanthocyanidins, a type of flavonoid is known for its high antioxidant properties. The antioxidant activity of the *L. camara* leaves reported is mainly attributed to the phenolics and proanthocyanidins, unlike the phenolics which has been often responsible for antioxidant properties. In addition, it has been reported that flavonoids from *Drynaria fortunei* plant have prevented nephrotoxicity, improved kidney function and promotes kidney primary epithelial tubular cell regeneration. However the flowers of *Lantana camara* is relatively virgin and pharmacological and phytochemical profile are incomplete. Keeping the literature review and hypothesis of antioxidant principles and organ protection in view the flowers of *Lantana camara* linn is selected for assessing the nephroprotective potential and its antioxidant role. So this study is essential and justifiable.

The flavonoids are polyphenolic compounds and reported to exhibit various pharmacological activities such as, cardiotonic activity, lipid lowering activity antioxidant activity, hepatoprotective activity, hypoglycemic activity etc and nephroprotective activity. In addition, it has been reported that flavonoids from *Drynaria fortunei* plant have prevented nephrotoxicity, improved kidney function and promotes kidney primary epithelial tubular cell regeneration. The antioxidant activity of the *L. camara* leaves reported is mainly attributed to the phenolics and proanthocynidines. Our literature survey revealed that the different part of *Lantana camara* such as flowers, roots, fruits and leaves have been screened for various pharmacological activities but there is no reports regarding the nephroprotective activity and antioxidant role of flowers of *Lantana camara* linn so far. So this study is essential and justifiable.

Chemical compounds isolated from extracts of leaves of *L. camara* are reported to have shown to exhibit antimicrobial, fungicidal, insecticidal and nematicidal activity. There are also reports that lantana compounds isolated from the extracts can be applied as weed killers and have been tested on the water hyacinth with some success. It is further reported that verbacoside, a compound isolated from lantana extract has been demonstrated to possess antimicrobial immunosuppressive and anti tumour activities. Use of lantana oil in treatment of skin itches and as an antiseptic for wounds and externally for leprosy and scabies is also reported.Use of lantana extracts in folk medicine for the treatment of cancers, chicken pox, measles, asthma, ulcers, swellings, eczema, tumors, high blood pressure, bilious fevers, catarrhal infections, tetanus, rheumatism, malaria and atoxy of abdominal viscera, is also reported. In this study we investigated the antimycobacterial activity of leaf extracts from *L. camara.*

It has several uses, mainly as an herbal medicine and in some areas as firewood and mulch. The leaves are used to relieve itching. Other uses are against flu, colds, coughs, fevers, yellow fever, dysentery and jaundice. The roots are used for gonorrhea. The use of lantana extracts as potential biocides have been suggested. Lantana oil is sometimes used for the treatment of skin itches, as an antiseptic for wounds and externally for leprosy and scabies. Lantana repels other plants and other groups of organisms such as insects. Lantana oil is used externally for leprosy and scabies. Plant extracts are used as medicine for the treatment of cancers, chicken pox, measles, asthma, ulcers, swellings, eczema, tumors, high blood pressure, bilious fevers, catarrhal infections, tetanus, rheumatism, malaria and atoxy of abdominal viscera.

Natural antioxidants such as α-tocopherol and L-ascorbic acid are widely used because they are seen as being safer and causing fewer adverse reactions, but their antioxidant activities are, however, lower than those of synthetic antioxidants such as butylated hydraxyanisole (BHA) and butylated hydraxytouluene (BHT). Hence, the need exists for safe, economic antioxidants with high activity from natural sources to replace these synthetic chemicals. The antioxidant compounds present in edible plants have recently been promoted as food additives because they display little or no toxic side effects.

The number of antioxidants' compounds synthesized by plants as secondary products, mainly phenolics, serving in plant defense mechanism to counteract ROS in order to survive, is currently estimated to be between 4000 and 6000 (11). A direct relationship has been found between the content of total phenolics and antioxidant capacity of plants (12). In fact, tocounteract deleterious action of ROS, phenolic compounds, naturally disturbed in plants, are effective. Because purified phenolic are difficult to obtain and because extracts sometimes have better antioxidant activity than those of pure molecules, there is a growing interest for the use of plant extracts. To find new natural sources of active compounds, we studied the antioxidant potential of different extracts of *L. camara*. The use of *Lantana camara* L. is listed as one of the important medicinal plants of the world. Lantana plants has been reported to possess a number of medicinal properties. Various parts of the plant are used in the treatment of itches, cuts, ulcers, swellings, bilious fever, catarrh, eczemea, dysentery and chest complaints of children, some metabolites isolated from their leaves have antitumor activity; inhibitor of protein kinase C; antimicrobial, antimotility, anti-inflammatory, insecticidal, and termicidal effects and antioxidant activity. Therefore the present study was undertaken to evaluate the wound healing property of *L. camara* leaves extract ethenolic leaf extract. Wound healing properties of *L. camara* also could be due to antimicrobial activity. *L. camara* extract exhibited significant antimicrobial activity and support folkloric use in the treatment of some disease as broad spectrum antimicrobial agents. This probably explains that these plants by the indigenous people against a number of infections since generation.

2.0 MATERIALS AND METHODS

1, 1 - Diphenyl – 2 - picrylhydrazyl was procured from Sigma-Aldrich India Company. Ascorbic acid, Gallic acid, Vanillin, Phloroglucinol, Ethyl acetate, Ethanol, Methanol, Acetone and Methanol were purchased from S. D. Fine Chemicals. All other

solvents are of AR grade and distilled before use. Distilled water was employed for all the experiments. Plant material was collected in the months of August – September in Tumkur district, Karnataka. The plant material was shade dried and powdered into 100 mesh size and stored at room temperature in an airtight container.

Extraction

50 g of *Lantana camara* powder was transferred into the clean and dry round bottom flask fitted with water cooled condenser. 300 ml of ethanol-water (80:20) was added to the flask and refluxed the contents for 3 hrs at 75^0 C by passing ice cold water through water cool condenser continuously. The contents were cooled, filtered, concentrated and dried to get dry extract. The extraction was repeated with methanol. Water and ethyl acetate to get extracts of respective solvents.
The extraction of the plant material was also performed with all the solvents mentioned above by soxhlet extraction method.

2.1 DPPH Assay

1,1 Dipheny 1-2 –picrylhydrazyl (Oxididized form) is a stable free radial with purple color in the presence of an antioxidant which can donate an electron to DPPH, the purple color which is typical to free DPPH radical dacays, and the change in absorbance at 520 nm is followed which can be measured spectrophotometrically. 39.4 mg of DPPH was dissolved in 100 ml methanol to get concerntration of 1mM stock and it was stored in dark bottle at 4 °C until further use. Methanol (50%) was prepared by diluting methanol 1:1 with de-ionized water Ascorbic acid standard stock I (conc.200µg/ml) was prepared by dissolving 2mg of ascorbic acid and make up to a volume of 10ml with de-ionized water. For making stander graph of ascorbic acid 2, 4, 6, 8, 10 µg/ml concentration range was used. The DPPH assay was carried out by using modified method Brand – Williams (13) in brief to a 860 µl of 50% methanol / ascorbic acid / test sample with various concentration, added 140µl of 1Mm DPPH, mixed and incubated at 37° C For 30 min. Read the observance at 520 nm against 50% methanol blank by spectrophotometer, a control reaction is carried out by without test sample addition. Color–correction contains the same concentration of the test sample in the methanol without DPPH. The anti-oxidant activity was measured with reference to the standard ascorbic acid observance values. The actual absorbance is taken as the absorbance difference of the control and the test sample and IC_{50} values were determined.

Antioxidants' which can inhibit or delay the oxidation of an oxidizable substrates in a chain reaction, therefore appear to be very important in the prevention of many diseases. Thus synthetic antioxidants are widely used in the food industry. However, because of their toxic and carcinogenic effects. Their use is being restricted. Thereby interest in finding natural antioxidants without undesirable side effects, has increased greatly.
Because purified phenolic compounds are difficult to obtain and because extracts sometimes have better antioxidant activities than those of pure molecules, there is a growing interest for the use of plant extracts. To find new natural sources of active compounds We studied the antioxidants potential of different extracts of *Lantana camara*.

2.2 Polyphenol Assay

Phenolic compounds in alkaline condition (sodium carbonate) dissociate to yield a proton and phenolate anion, which is capable of reducing Folin ciocalteu reagent (FC reagent). FC reagent is an oxidizing agent comprised of heteropolyphosphotungstate-molybdate. Sequence of one or two electron reduction reaction lead to blue color species. The blue coloured mixture of the 1-,2-,4-, and 6- electron reduction products in the tungstate series$P_2W_{18}O_{62}^{-7}$ $H_4P_2W_{18}O_{62}^{-8}$ to and the 2-,4- and 6-, electron reduction products in the molybdate series $H_2P_2Mo_{18}O_{62}^{-6}$ to $H_2P_2MO_{18}O_{62}$. Folin ciocalteu reagent (0.1N) was prepared by diluting 1:20 commercially available FC reagent with distilled water to get the required concentration. Sodium carbonate (7.5%) was prepared by dissolving 7.5 gm sodium carbonate in 100 ml of de-ionised water. Gallic acid (standard) stock 1 (conc.0.1mg\ml) was prepared by dissolving 1 mg of gallic acid in 10 ml with 50% methanol. For making standard graph of Gallic acid concentration range of 2-20µg/ml was used.The assay was carried out by using singleton, V. Rossi, J. A. Jr method (14), in brief, to a 200µL of 50% Methanol/standard/test sample with various concentration, 1000 µL of FC reagent was added, mixed and incubated at RT for 5 min. 800 µL of 7.5% sodium carbonate was added, mixed and incubated at RT for 30 min. The absorbance was recorded at 750 nm against blank by spectrophotometer, color correction was given with the same concentration was given with the same concentration of the test sample in 50%Methanol without FC reagent.

2.3 Flavonoids assay

Vanillin, an aromatic aldehyde condenses with the Flavon -3-ols and oligomers to form soluble pigments in acidic medium with an absorbance maximum at 500 nm, which can be detected by UV-Visible spectrophotometer. Vanillin reagent (1%) was prepared by dissolving 1gm of crystallized vanillin in 100 ml of 70% conc. H_2SO_4 (prepared fresh). Conc H_2SO_4 (70%) was prepared by diluting 70ml on conc. H_2SO_4 in 100 ml de-ionised water. Methanol 50% was prepared by diluting 1:1 with de-ionised water.
Phloroglucinol (standard) stock 1 (1 mg/ml): 10 mg of phlorogluinol was dissolved and made up to volume of 10 ml with 50% Methanol, Then centrifuge at 12,000 rpm for 10 min. The original stock was diluted to to yield 0.1mg/ml with 50% methanol. For making standard graph of phloroglucinol, 1-10 µg/µL concentration range was used. The flavonoid assay was carried out by using Swain, T and Hillis, W. E method (15). In brief, to a 400µL of distilled water/ positive control/ test sample with various concentration, added 800 µL of 1% vanillin reagent, mixed and incubated at RT 15 minutes. Read the absorbance at 500nm against blank by spectrophotometer. Colour correction was given with the same concentration with the test sample in distilled water without vanillin reagent. The flavonoids content in the phyto-extracts was measured with reference to the standard Gallic acid values.

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfer electron from a substance to an oxidizing agent Oxidation reaction can produce free radicals. In turn these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death. When the chain reaction occurs in a purified monomer, it produce a polymer resin, such as a plastic, a synthetic fiber, or an oil paint film, Antioxidant terminate these chain reaction by removing free radical intermediates and inhibit other oxidation reaction. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols.
Although oxidation reaction are crucial for life, they can also be damaging: hence plants and animals maintain complex systems of multiple types of antioxidants such as glutathione, vitamin C and vitamin E as well as enzymes such as catalase superoxide dismutase and various super oxidases. Low level of antioxidants or inhibition of the antioxidants enzymes, cause oxidative stress and damage or kill cells.
As oxidative stress appears to be an important part of many human diseases the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. However, it is unknown whether oxidative stress is the cause or the consequence of disease.

3.0 RESULTS AND DISCUSSION

Free radicals were a major interest for early physicists and radiologists and much later found to be a product of normal metabolism. Although oxygen is essential for aerobic forms of life, oxygen metabolites are highly toxic. As a consequence, reactive oxygen species are known to be implicated in many cell disorders and in the development of many diseases including cardiovascular diseases, atherosclerosis, cataracts, chronic inflammation, and neurodegenerative diseases. ROS and free radicals are also considered as inducers of lipid peroxidation and cause the deterioration of foods. Although organisms have endogenous antioxidant defenses produced during normal cell aerobic respiration against ROS, other antioxidants are taken from the diet, both from natural and synthetic origin

An antioxidant is a capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electron from a substance to an oxidizing agent. Oxidation reaction can produce free radicals. In turn, these radicals can start chain reaction. When the chain reaction occurs in a cell, it can cause damage or death. When the chain reaction occurs in a purified manner, it produces a polymer resin, such as a plastic, a synthetic fiber, or an oil paint film. Antioxidants terminate these chain reactions by removing free radical intermediates are inhibit other oxidation reaction. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols.
Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidants enzymes, cause oxidative stress and may damage or kill cells.

As oxidative stress appears to be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly as treatment for stroke and neurodegenerative disease. However, it is unknown whether oxidative stress in the cause or the consequence of disease. Antioxidants are widely used as ingredients in dietary supplements and have been investigated or the prevention of disease such as cancer coronary heart and even altitude sickness. Although initial studies suggested that antioxidant supplements might promote health, later large clinical trials did not detect any benefit and suggest instead that excess supplementation is harmful. In addition to these uses of natural antioxidants' in medicine, these compounds have many industrial uses such as preservatives in food and cosmetics and preventing the degradation of rubber and gasoline.
Antioxidants, which can inhibit or delay the oxidation of an oxidizable substrate in a chain reaction, therefore, appear to be very important in the prevention of many disease, Thus, synthetic antioxidants are widely used in the food industry. However, because of their toxic and carcinogenic effects, their use is being restricted. Thereby, interest in finding natural antioxidants, without undesirable side effects greatly. Because purified phenolic compounds are difficult to obtain and because extracts sometimes have better antioxidant activities than those of pure molecules, there is a growing interest for the antioxidant potential of different extracts of *L. camara.*

3.1 Extraction yields:

Leaves reflux extraction:

Sl. No.	Solvent	% yield
01	Water	8.4%
02	80% alcohol	7.6%
03	Ethyl acetate	12.4%
04	Methanol	8.22%

Leaves Soxhlet extraction:

Sl. No.	Solvent	% yield
01	Water	5.84%
02	Ethanol	5.2%
03	Ethyl acetate	7.1%
04	Methanol	7.35%

Stem reflux extraction:

Sl. No.	Solvent	% yield
01	Water	7.72%
02	Ethanol	4.4%
03	Ethaylacetate	4.59%
04	Methanol	7.84%

Stem soxhlet extraction:

Sl. No.	Solvent	% yield
01	Water	4.97%
02	80% alcohol	3.41%
03	Ethaylacetate	4.30%
04	Methanol	5.91%

Root reflux extraction:

Sl. No.	Solvent	% yield
01	Water	4.67%
02	80% alcohol	4.25%
03	Ethaylacetate	4.2%
04	Methanol	5.24%

Root soxhlet extraction:

Sl. No.	Solvent	% yield
01	Water	4.08%
02	80% alcohol	4.00%
03	Ethaylacetate	2.48%
04	Methanol	4.26%

The solvent such as water, ethanol, ethyl acetate, Methanol were utilized optimum extraction process so as to arrive at extracts with higher yield and better antioxidant potency. It was observed that Methanol is the solvent which provides a highest yield among all the solvents.

3.2 Polyphenol content:

Leaves:

Sl. No.	Solvent	Polyphenol content
01	Water	16.9%
02	Ethanol	28.57%
03	Ethyl acetate	12.5%
04	Methanol	31.2%

Stem:

Sl. No.	Solvent	Polyphenol content
01	Water	14.3%
02	Ethanol	19.2%
03	Ethyl acetate	15.87%
04	Methanol	18.18%

Root:

Sl. No.	Solvent	Polyphenol content
01	Water	66%
02	Ethanol	21.7%
03	Ethyl acetate	14.4%
04	Methanol	20.20%

Polyphenol content was determined by singleton method. It was found that the ehtanolic and methanolic extracts were found to have highest percent of polyphenols.

3.3 Flavonoid content:

Leaves:

Sl. No.	Solvent	Flavonoid content
01	Water	29.4%
02	Ethanol	66%
03	Ethyl acetate	27.02%
04	Methanol	32%

Stem:

Sl. No.	Solvent	Flavonoid content
01	Water	33.08%
02	Ethanol	33%
03	Ethyl acetate	10.5%
04	Methanol	40%

Root:

Sl. No.	Solvent	Flavonoid content
01	Water	38%
02	Ethanol	14.49%
03	Ethyl acetate	11.9%
04	Methanol	64%

It is evident from above data that methanol extracts is proved to have highest contents of flavonoids.

3.4 DPPH Activity:

Leaves:

Sl. No.	Solvent	IC_{50} Values
01	Water	130 µg/ml
02	Ethanol	260 µg/ml
03	Ethyl acetate	350 µg/ml
04	Methanol	290 µg/ml

Stem:

Sl. No.	Solvent	IC_{50} Values
01	Water	320 µg/ml
02	Ethanol	420 µg/ml
03	Ethyl acetate	360 µg/ml
04	Methanol	210 µg/ml

Root:

Sl. No.	Solvent	IC_{50} Values
01	Water	360 µg/ml
02	Ethanol	650 µg/ml
03	Ethyl acetate	110 µg/ml
04	Methanol	250 mg/ml

DPPH a stable free radical with a characteristic absorption at 517-520 nm was used to study radical scavenging effects of extracts. Inhibition of DPPH activity was shown to be maximum with water extract in the case leaves, methanol extract in case of stem and ethyl acetate in case of root. It is clear from the DPPH Activity studies that various extracts of different parts have significantly higher DPPH Inhibitory activities.

4.0 CONCLUSION

The present investigation deals with the extraction, phytochemical constituent analysis and antioxidant activity analysis. It was observed that the yields of extraction depend on nature of solvent. It was found that the ethanol and methanol are the best possible solvents for having higher yields compared to other solvents chosen of varied polarities. Polyphenol content determination indicates that the ethanol and methanol extracts were found to have highest percent of polyphenols than other extracts. However, the other extracts also contain considerable amounts of the polyphenols. This indicates that all these extracts may have significant antioxidant activities thereby eliciting beneficial physiological effects. The Flavonoid estimation of the extracts indicate that methanol extract possess highest amount of flavonoids among all the extracts of different solvents. Other extracts also have substantial amounts of flavonoids. Since the flavonoid content is directly proportional to the antioxidant activity, these extract could exhibit potential antioxidant activities. The DPPH activity analysis indicates that water extract evidenced to have potential antioxidant activity among all extracts of different solvents. This is probably due to the significantly high flavonoid content compared to other extracts. It is also clear that *L. camara* seems to have highly soluble compounds in ethanol and methanol; hence the yield is also high.

Unequivocally, water extract emerges out as the best possible extract in terms of antioxidant activity. But methanol, ethyl acetate and ethanol extracts are shown to high higher yields. However, methanol and ethyl acetate are not suitable solvents for consumption and extraction as they may cause severe deleterious effects even at very low concentrations. Due to this, the regulatory bodies all across the globe have very stringent norms for using methanol and ethyl acetate as solvents of extraction. In lieu of these facts, the comparatively potent water and ethanol extract could be utilized as potential antioxidant extracts. The *Lantana camara* has extensively been used in most of the countries across the globe and especially in Indian subcontinent even though it has been widely used in India since very long time, its health beneficial effects have not been documented scientifically until recently. However for the past few years considerable number of studies has attempted to understand its nature and its bioactivity. This investigation points out that the *Lanatana camara* contains significant amounts of antioxidant compounds such as polyphenols and flavonoids. Consequently various extracts showed potential antioxidant activity. Hence it is worth to consider *Lantana camara* for further exploration in utilizing it as rich antioxidant.

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