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Study Of Antimicrobial And Phytochemical Analysis Of Solvent Extracts Of *Lantana Camara* Leaves Against Pathogenic Microbes

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Abstract
Lantana camara is a widely distributed shrub in tropical and subtropical regions and has been recognized for its medicinal properties in traditional medicine systems. This study aimed to focus and explore the antimicrobial potential and phytochemical composition of *Lantana camara* leaves extracts. Various solvents including ethanol, ether, benzene and water were used for extraction. Antimicrobial activity was assessed against pathogenic microorganisms including bacteria using agar well diffusion method. Phytochemical analysis was done to identify the presence of bioactive compounds using standard procedures. The results showed significant antimicrobial activity of *Lantana camara* extracts against pathogens such as *Staphylococcus aureus and Escherichia coli.* The ethanolic and ether extracts were found to be more effective in comparison to other extracts. The phytochemical analysis of this study showed the presence of various secondary metabolites including alkaloids, flavonoids, tannins and terpenoids etc which are known for their antimicrobial properties. The potential of *Lantana camara* as a natural source of antibacterial compounds is highlighted by this study to understand the active ingredients responsible for antimicrobial activity. Further studies are however needed to explore and identify such molecules.

Keywords: Antibacterial, benzene, phytochemical, solvent extraction.

INTRODUCTION

In many cultures, using medicinal herbs for therapeutic purposes is a secular practice. Vegetable species have demonstrated efficacy in treating a variety of illnesses, including cancer (Navvaro et al., 2005), because of the biodiversity of Brazilian plants and the fact that some microorganisms develop resistance to traditional antibiotics, scientists have focused on natural products as a source of novel compounds with pharmacological potential that may be less harmful to human health and more effective against nosocomial infection (Ferronato et al., 2007 and Salvagnini et al., 2008).

Lantana camara is a widely distributed shrub in tropical and subtropical regions and has been recognized for its medicinal properties in traditional medicine systems. Native to tropical America, L. camara is a short, erect, rough, hairy, evergreen shrub (Verbenaceae). Throughout the world, this major weed—known by several common names such as black sage, cuasquito, angel lip, flowered sage, shrub verbena, white sage, and wild sage. It is found in more than 60 countries and island groupings, with approximately 650 variations. *L. camara* serves a variety of purposes, mostly as an herbal remedy and, in certain regions, as mulch and fuel. Cancer, chicken pox, measles, asthma, ulcers, swellings, eczema, tumors, high blood pressure, bilious fevers, catarrhal infections, tetanus, rheumatism, malaria, and atoxy of the abdominal viscera are among the conditions for which it is also utilized. (Abu-Shanab et al.,2006).

There have been reports of *L. camara's* antiviral, antifungal, antibacterial, antiprotozoal, and antinematode properties. There have been reports of antibacterial activity in *L. camara* nanoparticles, EOs, and solvent extracts. Nearly all biological activity in *L. camara* is thought to be attributed to the presence of lantadenes. The biological activities were also discovered to be attributed to other minor elements found in leaf EOs, such as E-nerolidol, bicyclogermacrene, and pinene, as well as constituents like 1,8-cineole, sabinene, and caryophyllene. The antibacterial qualities of it may be attributed to the presence of proanthocyanidins, anthocyanins, and phenolics in its leaves (Ganjewala et al., 2009) The permeability barrier of cell membrane structures is disrupted by the extracts active principle, which prevents bacterial growth (Priya and Ganjewala, 2007).

Chemical components

The foundation of many pharmaceutical enterprises is derived from phytochemical elements, which are also crucial to the identification of unprocessed pharmaceuticals. These plants have medical potential because they contain certain chemicals that have a specific physiological effect on humans. When compared to the often-used synthetic chemotherapeutic drugs, the most significant characteristic of these bioactive plant elements is their increased effectiveness with negligible or no side effects. Their high levels of steroids, tanning, terpenoids, and saponins are responsible for their anti-inflammatory, antispasmodic, and anti-analgesic properties. (Mallikaharajuna et al., 2007)

Bioactive substances such as flavones, isoflavones, flavonoids, anthocyanins, coumarins, lignans, catechins, isocatechins, alkaloids, tannin, saponins, and triterpenoids are abundant in *L. camara*. A few writers have assembled the

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information regarding the phytochemistry of L. camara, based on reports of the numerous bioactive compounds that were isolated from various plant parts and its essential oils (Ghisalberti 2000, Sharma et al., 2013).

Therapeutic uses

The use of plant is in herbal medicine. The leaves of this plant are used for wound healing, fever treatment, cough treatment, influenza treatment, stomach ache, malaria. A tea prepared from the leaves and flowers of lantana is good against different diseases and fever. It possesses anti-inflammatory properties, which may help reduce inflammation in the body.It helps in reducing anxiety and memory impairment.

The purpose of this study was to evaluate the *in vitro* antibacterial activity and phytochemical screening of different extracts from *L. Camara* leaves using well diffusion method.

MATERIALS AND METHODS

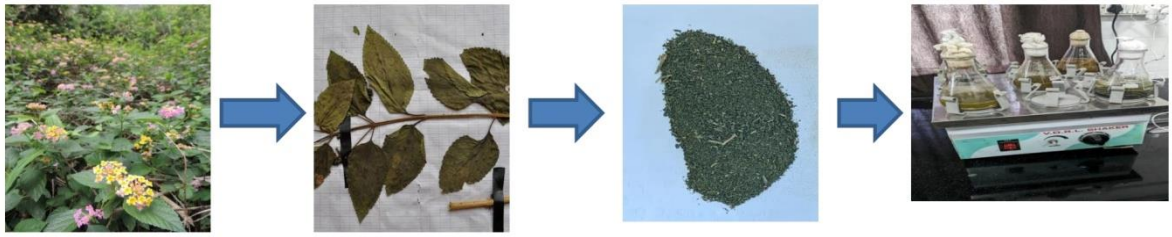

Figure 1: Plant collection and extract preparation

Leaves of *Lantana camara* were procured in February 2024 form Kimadi forest range, Uttarakhand. The plant material was washed thoroughly to remove any dirt or contaminants. The plant material was shade dried and grinded into fine powder using a mortar and pestle or a grinder.

Extract Preparation

10gm of the powder was weighed and mixed with 100 ml of solvent (Distilled water, Ether, Ethanol and Benzene). The mixture was kept in a conical flask, with its mouth completely sealed; on a shaker for 48 hours. The resulting product was then filtered. Filtrate was then evaporated in a water bath for obtaining the extract which was further mixed in DMSO.

Development and Maintenance of Test Microorganism for Antibacterial Studies Bacterial cultures such as *Escherichia coli* and *Staphylococcus aureus* were obtained from Research Laboratory, SGRR University, Dehradun. These bacteria were maintained in Nutrient Broth at 37^0C.

Phytochemical screening of leaves extract

A part of the extracts was used to identify some of the secondary metabolites such as alkaloids, flavonoids, tannins, saponins, protein carbohydrates etc. based on the following procedures given by Evans, 2009.

Test for Tannins: - 0.5ml of extract was added to 1ml ferric chloride. Brownish black color indicates positive test.
Test for Saponins: - 5.0ml of distilled water was mixed with aqueous crude plant extract in a test tube and it was mixed vigorously. Frothing was mixed with few drops of olive oil and mixed vigorously and the foam appearance showed the presence of saponins.
Test for Flavonoids: 0.5 ml 0f extract is taken and 0.25ml of lead acetate is added to it. Positive test is obtained by appearance of white color on top.
Test for Protein (Biuret test): - 2ml of filtrate was taken to which 1 drop of 2% copper sulphate solution was added; 1ml of 95% ethanol was added. Then it was followed by excess addition of KOH. The appearance of pink colour indicates the presence of protein.
Test for carbohydrates (Molisch test): - 1ml of leaf extract was added to 2ml of leaf extract and a few drops of H2SO4 were added. Purple or reddish color gives a positive test.
Test for Alkaloids (Mayers Test): - 1ml of each extract was taken and few drops of Mayers reagents were added in each test tube, appearance of white creamy colour indicates the presence of alkaloids.
Test for Amino acid (Ninhydrin test): - 1ml of each extract was taken and two drops of Ninhydrin reagents were added in each test tube. Appearance of purple colour indicates the presence of amino acids.
Test for Terpenoids (Salkowski test): -2ml of each extract was taken and in 1 ml of chloroform ,2 ml extract was added along with few drops of conc.H_2SO_4. Formation of reddish brown precipitate indicates the positive result.

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Test for Fats and oils (Spot test): - Small quantity of each extract was taken on filter paper, after 5 min filter paper was observed. Oil stain on the paper indicates the presence of fats and oils.
Test for Glycoside compounds (Keller Killani test): -1 ml of glacial acetic acid was added to 1 ml of plant extract samples and cooled. After cooling, 2 drops of $FeCl_3$ was added followed by careful addition of conc.H_2SO_4 along the walls of the test tube. Reddish brown colour ring formed at the junction of two layers indicated the existence of glycosides. (Archana et al., 2012).

Evaluation of antibacterial activity
Individual *L. camara* leaf extracts at three different concentrations (10µl, 20µl & 30µl) were screened for in vitro antibacterial activity against *Staphylococcus aureus* and *E.coli* and compared with standard antibiotic streptomycin (Positive Control) using agar well diffusion method.

Agar well diffusion method
The nutrient agar medium was made and autoclaved for 20 minutes at 121°C to sterilize it. After that, the prepared media was added to a Petri plate and left to solidify. Using sterile swabs, test bacterial cultures that were one day old were uniformly swabbed on NA medium and left to stand for thirty minutes. Using a sterile cork borer, wells were created on previously inoculated agar plates. Wells were created in each plate. Using micropipettes, each extract was made into a distinct dilution and applied to the wells. Following that, the plates were incubated for 24 hours at 37°C. The zone of inhibition was measured and observed following incubation (Rajashekar et al., 2013; Rizvi et al., 2013).

RESULTS

Phytochemical analysis of leaves of *Lantana Camara*
The results of qualitative phytochemical screening of four-leaf extracts are shown in **Table 1** and it was observed that maximum phytochemical constituents were present in benzene extract followed by extracts prepared in ethanol, ether and distilled water.
Out of ten phytoconstituents, eight showed positive results in benzene extract which were identified as Alkaloids, flavonoids, Tannins, carbohydrates, glycosides, terpenoids, proteins and oil and fats whereas in other three extracts alkaloids, tannin and carbohydrate, glycosides, terpenoids, proteins and oil and fats were present.

Antibacterial assay
When the interaction between bacteria and tested solvent extract (BXS) was studied, significant values were observed with ether extract which showed maximum antibacterial activity against *S. aureus* at 30 µl(ZOI:19.3mm) and *E.coli* (ZOI:21mm) followed by ethanol extract For *S. aureus* (ZOI:17.6mm) and 21 mm zone of inhibition for *E.coli*. Benzene extract showed less ZOI in both case whereas aqueous extract showed no significant zone of inhibition in case of both *S. aureus* and *E.coli* **(Table 2 and Table 3; Figure 1 -5).**

Table: 1- Phytochemical analysis of leaf extract of *L. camara*

Phytochemicals	**Test**	**Leaf Extract**			
		Ethanol	**Benzene**	**Ether**	**Distilled Water**
Alkaloids	Wagner's test	+	+	+	+
Flavonoids	Alkaline reagent test	-	+	-	-
Saponins	Chloroform & H2SO4 test	-	-	-	-
Tannins	Ferric chloride test	+	+	+	+
Amino acid	Ninhydrin	-	-	-	-
Carbohydrate	Benedict's	+	+	+	+
Glycoside	Keller Killani test	+	+	+	+
Protein	Biuret	+	+	+	+
Terpenoid	Salkowski test	+	+	+	+
Oil & Fats	Spot test	+	+	+	+

Table: 2- Zone of Inhibition of *Staphylococcus aureus*

S.NO.	**Solvent**	**Concentration (µl)**	**R1**	**R2**	**R3**	Mean
1.	Ether	10	14.5	14	13	13.8
		20	17	19	18	18
		30	20	19	19	19.3
2.	Ethanol	10	14	13	14	13.6
		20	18	19	16	17.6
		30	18	18	16	17.6

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3.	D.W.	10	NA	NA	NA	NA
		20	NA	NA	NA	NA
		30	NA	NA	NA	NA
4.	Benzene	10	13	12	12.5	12.5
		20	15	17	16	16
		30	16	17	14	15.6

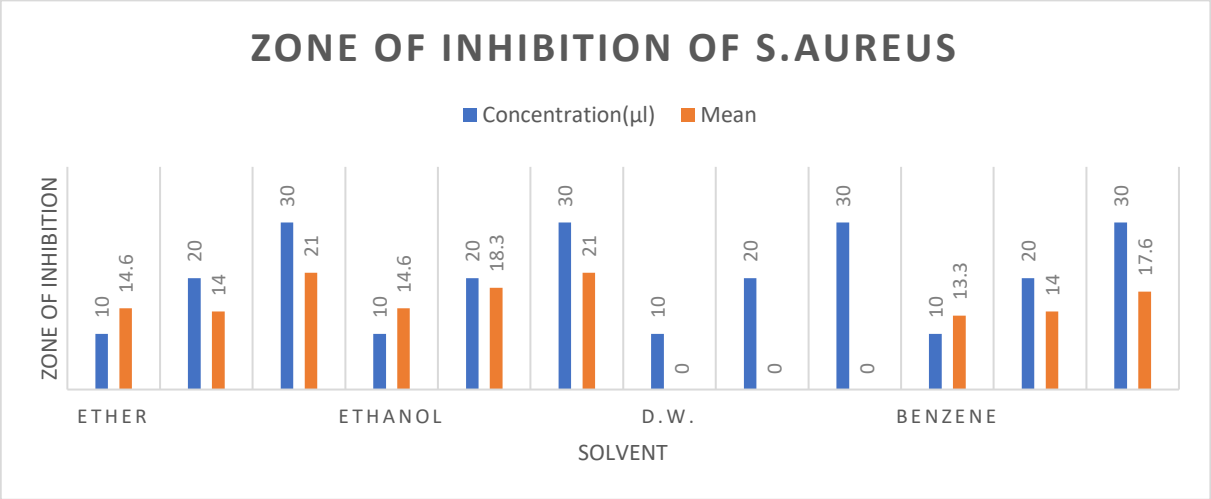

Figure 1: Zone of Inhibition against S. aureus

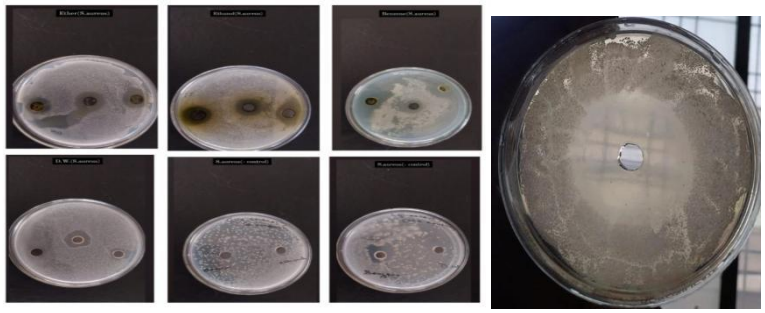

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Figure: 2- Antibacterial activity of different extracts of Leaves of *L. camara* against *S.aureus*

Table 3- Zone of Inhibition of *E.coli*

S.NO.	**Solvent**	**Concentration(µl)**	**R1**	**R2**	**R3**	Mean
1.	Ether	10	13	16	14	14.6
		20	14	15	13	14
		30	21	22	20	21
2.	Ethanol	10	15	14	15	14.6
		20	18	18	19	18.3
		30	21	20	22	21
3.	D.W.	10	NA	NA	NA	0
		20	NA	NA	NA	0
		30	NA	NA	NA	0
4.	Benzene	10	13	15	12	13.3
		20	15	13	14	14
		30	18	17	18	17.6

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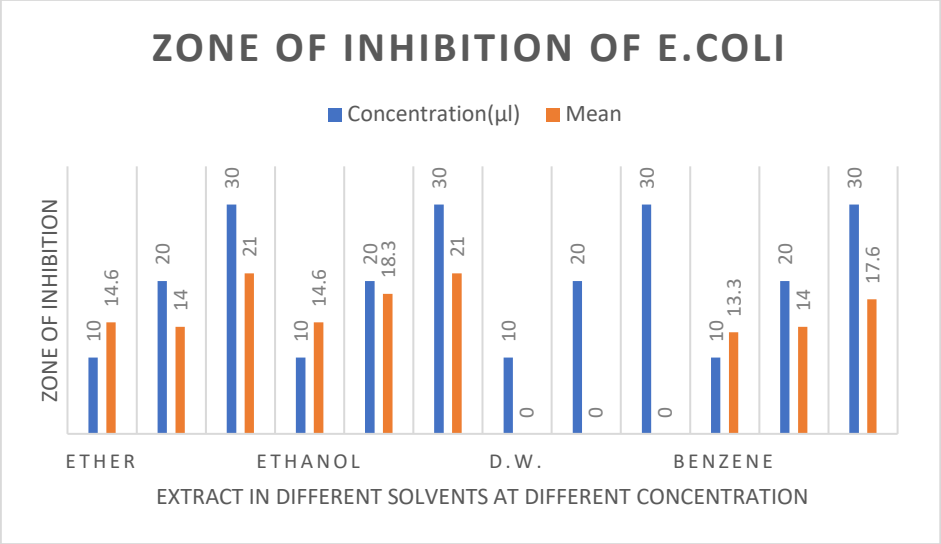

Figure 3- Graphical representation of ZOI of *E.coli*

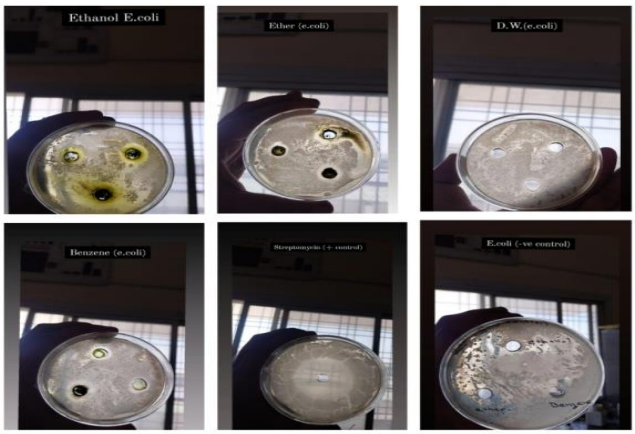

Figure: 4- Antibacterial activity of different extracts of Leaves of *L.camara* against *E.coli*

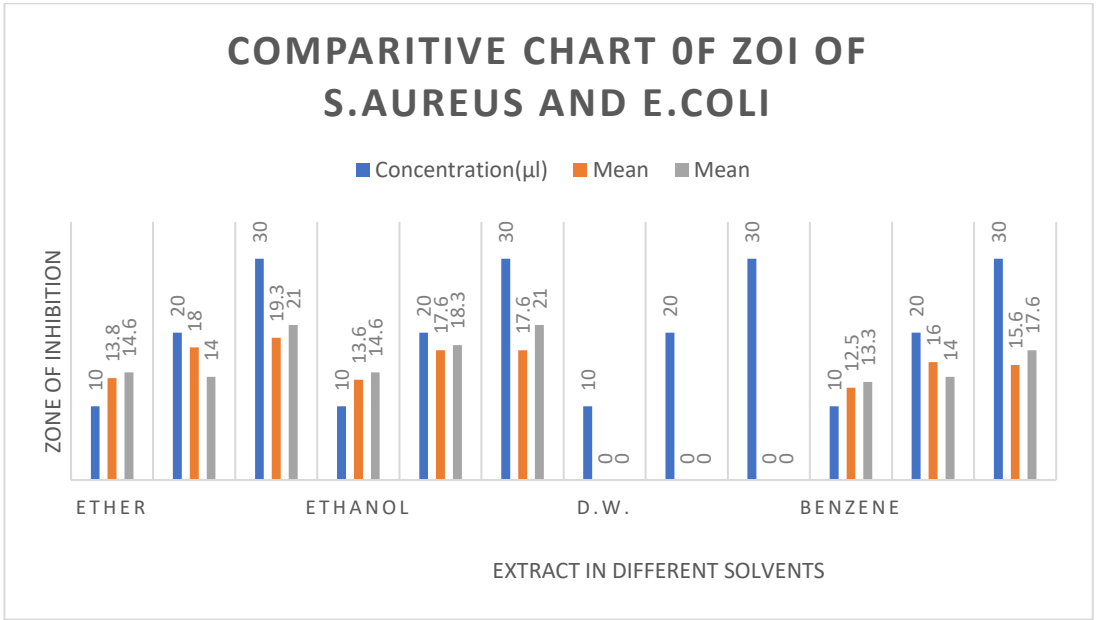

Figure 5- Comparison of ZOI of *S. aureus and E. coli*

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Discussion

The antibacterial qualities of *L. camara* have been the subject of much research (Mello et al., 2005; Verma & Verma, 2006). It is involved in numerous significant biological processes. Nearly all biological activity in it is thought to be attributed to the lantadenes found in the plant (Barre et al., 1997). Furthermore, some of these biological actions may also be partially attributed to other secondary metabolites such phenolics, terpenoids, and alkaloids (Barre et al., 1997). However, it was also shown that the biological actions of essential oils were caused by compounds found in leaf essential oils, such as 1, 8-cineole, sabinene, and caryophyllene, as well as other minor constituents including E-nerolidol, bicyclogermacrene, and pinene (Sonibare& Effiong, 2008).

Compared to Gram negative bacteria, the extracts from L. camara exhibited a wider range of inhibitory effect against Gram positive bacteria. Nevertheless, it was discovered that Staphylococcus aureus was resistant to the extract (Ganjewala et al., 2009). These leaf and floral extracts exhibit antifungal and antidermatophytic properties as well. The most effective extract of leaves and flowers in terms of fighting fungi and dermatophytes is chloroform.

In this study, *Lantana camara*, leaf extract showed potential antibacterial activity against various clinically relevant gram-negative and gram-positive bacterial species. Moreover, *L. camara* ether extract had the best antibacterial activity compared to the other solvents used in the current study. The ether extract exhibited high activity against S. *aureus compared to E.coli*. This finding agrees with that of Barre et al., who used the extract of *L. camara* to inhibit the growth of pathogen *S. aureus* owing to the presence of active phytocompounds possessing bactericidal effects. Gram-positive bacteria were more sensitive to *L. camara* ether and ethanolic extract than gram-negative bacteria (*E. coli*).

Conclusion

This study showed that ether and ethanolic extracts of leaves of *Lantana camara*, have much potential to inhibit the growth of various gram-negative and gram-positive bacterial species. The above-mentioned findings could be a potential topic for further in vivo studies to develop therapeutic plant extracts from *Lantana camara* and avoid the health hazards and antimicrobial resistance associated with the use of chemically synthesized antimicrobial agents. The studies final finding validates the use of several plant extracts in Indian traditional medicine to treat various diseases brought on by pathogenic bacteria. This investigation demonstrated the presence of phytochemical substances with antibacterial properties in *L. camara*. Furthermore, *L. camara's* leaf extract in ether and ethanol have been shown to be effective against tested pathogenic microbes. It also implies that, while looking at natural food and feed additives to improve human and animal health, a great deal of attention should be made to medicinal plants, which are shown to contain plenty of pharmacological qualities that may be sufficiently better.

References

1. Abu-Shanab B, Adwan G, Jarrar N, Abu-Hijleh A, Adwan K. Antibacterial activity of four plant extracts used in Palestine in folkloric medicine against Methicillin-resistant Staphylococcus aureus. Turk. J. Biol 2006; 30: 195-4.
2. Adiguzel A, Gulluce M, Sengul M, Ogutcu, Sahin, F. Karaman I.. Antimicrobial effects of Ocimum basilicum (Labiatae) Extract. Turk. J. Biol 2005; 29: 155-6.
3. Agarwal GP, Hasija SK. In Agarwal GP, Hasija SK, editors. Microorganisms in the laboratory guide of Microbiology, Mycology and Plant Pathology: CBS publishers Lucknow (U.P); 1986. 1- 48.
4. Archana P, Samatha T, Mahitha B, Chamundeswari NR. Preliminary phytochemical screening from leaf and seed extracts of *Senna alata* L. Roxb- an ethno medicinal plant. *Int J Pharm Biol Res.* 2012;**3**:82–89.
5. Barre, JT, Bowden BF, Coll JC, Jesus JDe, De La Fuente VE, Janairo GC, Ragasa CY. A bioactive triterpene from Lantana camara. Phytochem 1997; 45(2):321-5.
6. Begum S, Wahab A, Siddiqui BS. Ursethoxy acid, a new triterpene from Lantana camara. Nat. Prod. Lett. 2002; 16(4): 235-8.
7. Cobos MI, Rodriguez JL, Oliva ML, Demo M, Faillaci SM, Zygadlo, JA Composition and antimicrobial activity of essential oil of Baccaris notosergila. Planta Med 2001; 67(1): 84-6.
8. Evans WC. (2009). Trease and Evans Pharmacognosy. 16th Edition. Saunders Publishers, London. pp. 42–44, 221–229.
9. Ferronato R, Marchesan ED, Pezenti E, Bednarski F, Onofre SB. *Rev Bras Farmacogn.* 2007. Atividadeantimicrobiana de óleosessenciaisproduzidospor *Baccharis dracunculifolia* DC. e *Baccharis uncinella D.C.* (Asteraceae) p. 224.p. 30.
10. Ganjewala D, Sam S, Khan KH. Biochemical compositions and antibactericidal activities of *Lantana camara* plants with yellow, lavender and white flower. Eur Asian J Biosci 2009; 3(10):69-77.
11. Ghisalberti EL. *Lantana camara* L. (Verbenaceae). Fitoterapia 2000;71:467-86.
12. Grover RK, Moore JD. Toximetric studies of fungicides against the brown rot organisms, Sclerotinia fructicola and S. laxa. Phytopathol 1962; 52: 876-4.
13. Leite SP, Vieira JRC, Medeiros PL, Leite RMP, Lima VLM, Xavier HS, Lima EO. Antimicrobial Activity of Indigofera suffruticosa. Evid Based complement Alternat Med 2006; 2:13- 5.

14. Mallikaharajuna PB, Rajanna LN, Seetharam YN, Sharanabasappa GK. Phytochemical studies of Strychnospotatorum L.F. -A medicinal plant. EJ Chem. 2007; 4:510-518.
15. Mazumder A, Saha BP, Basu SP, Mazumder R. A comparison of in vitro antifungal activity of the Methanol extract of various parts of Lagerstroemia parviflora (Roxb). Ethiop. Pharm. J 2004; 22: 61-5.
16. Mello F. B., Jacobus D., Carvalho K and Mello J. R. B. Effects of *Lantana camara* (Verbenaceae) on general reproductive performance and teratology in rats. *Toxicol. Res*. 2005;45:459-466.
17. Moshi MJ, Mbwambo ZH. Some pharmacological properties of extracts of Terminalia sericea roots. J. Ethnopharmacol 2005; 97(1):43-5.
18. Navarro D. *Tese de Doutorado. Universidade Federal de Santa Catarina.* Florianópolis, SC: 2005. Estudoquímico, biológico e farmacológico das espécies *Allamanda blanchetti* e *Allamanda schottii* Pohl para a obtençõo de frações e moléculasbioativas de potencialterapêutico.
19. Oliveira IS, Lima JC, Silva RM, Martins DT. Triagem da atividadeantibacteriana *in vitro* do látex e extratos de *CrótonurucuranaBaillon*. *Rev Bras Farmacogn.* 2008;18:587–593.
20. Priya K, Ganjewala D. Antibacterial activities and phytochemical analysis of different plant parts of *Nyctanthesarbor-tristis* L. Res J Phytochem 2007;1: 61-7.
21. Rajashekar Y., Kumar H.V., Kothapalli V.R., Bakthavatsalam N., "Isolation and characterization of biofumigant from leaves of Lantana camara for control of stored grain insect pests", Industrial Crops and Products, Vol. 51, pp. 224–228, 2013.
22. Rizvi Z.F., Mukhtar R., Chaudhary M.F., Zia M., "Antibacterial and Antifungal Activities ofLawsoniainerm is, Lantana camara and Swertiaangustifolia", Pakistan Journal of Botany, Vol. 45, no. 1, pp. 275–278, 2013.
23. Salvagnini LE, Oliveira JR, Santos LE, Moreira RR, Pietro RC. Avaliação da atividadeantibacteriana de folhas de *Myrtus communis L.* (Myrtaceae) *Rev Bras Farmacogn.* 2008;18: 241–4.
24. Satish S, Raveesh KA, Janardhna GR. Antibacterial activity of plant extracts on phytopathogenic Xanthomonas campestris pathovars. Lett. Appl. Microbiol 1999; 28:145-3.
25. Sharma OP, Makkar HPS, Dawra RK. A review of the noxious plant Lantana camara. Toxicon 1988; 26: 975-13.
26. Sharma P, Shrivastava B, Sharma GN, Jadhav HR. Phytochemical and pharmacological profile of *Lanata camara*: An overview. J Adv Pharm Educ Res 2013; 3(4):294-305.
27. Sonibare O and Effiong I. Antibacterial activity and cytotoxicity of essential oil of *Lantana Camara* leaves from Nigeria. *African J. Biotechnol*. 2008;7(15):2618-2620.
28. Verma R. K and Verma S. K. Phytochemical and termiticidal study of *Lantana camara* aculeata leaves. *Fitoterapia*. 2006; 77(6):466-468.
29. Wuthi-udomlert M, Prathanturarug S, Soonthornchareonnon N. Antifungal activities of Senna alata extracts using different methods of extraction. International conference on Medicinal and Aromatic plants. 1998; 26:1-61.