RESEARCH ARTICLE ISSN: 2349-2678

Contents lists available at www.ijpba.in
International Journal of Pharmaceutical and Biological Science Archive
NLM (National Library of Medicine ID: 101738825)
Index Copernicus Value 2019: 71.05
Volume 9 Issue 2; March-April; 2021; Page No. 14-23

PHYTOCHEMICAL SCREENING AND ANTIACNE ACTIVITY OF LEAVES EXTRACT OF *LAGERSTROEMIA INDICA*

Ms. Vandana Vishwakarma*, Mr. Narendra Patel, Dr. Prabhakar Budholiya

College of Pharmacy, Sri Satya Sai University of Technology and Medical Sciences, Sehore (M. P.)

Conflicts of Interest: Nil

Corresponding author: Ms. Vandana Vishwakarma

DOI: https://doi.org/10.32553/ijpba.v9i2.184

ABSTRACT

Medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing. Secondary constituents contain alkaloids, flavonoids, phenol, sapon in, steroids and tannins. Medicinal plants have anticancer, antimicrobial, antidiabetic, antidiuretic and anti-inflammation activities. The increasing interest in powerful biological activity of secondary metabolites outlined the necessity of determining their contents in medicinal plants. In Indian Ayurvedic system, *Lagerstroemia Indica* (Lythraceae) are well-known plants used for major and minor ailments. The aim of the present study is to examine leaf of *Lagerstroemia Indica* for phytochemical profile, antioxidant potential, antimicrobial including anti acne activity. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics and flavonoids were determined by the well-known test protocol available in the literature. Quantitative analysis of phenolic and flavonoids was carried out by FolinsCiocalteau reagent method and aluminium chloride method respectively. Phytochemical analysis revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids, fixed oil and fats. The present study concluded that the crude extract of *Lagerstroemia Indica* is a rich source of secondary phytoconstituents which impart significant antioxidant potential. The extract of *Lagerstroemia Indica* possess antimicrobial effectiveness againt P. acne and other microbes, In Vitro anti acne activity. The findings of the present study will be helpful to phytochemists, pharmacologists and pharmaceutical industries.

Keywords: *Lagerstroemia Indica*, Phytochemical, Antioxidant, Antiacne.

1. INTRODUCTION

1.1 ACNE

The word **acne** comes from the word *acme* meaning "the highest point", which comes from the Greek word *akme* meaning "point" or "spot". It was originally mispelt, with an 'n' rather than an 'm' in 1835. Acne, medically known as *Acne vulgaris*, is a skin disease that involves the oil glands at the base of hair follicles. It commonly occurs during puberty when the sebaceous (oil) glands come to life and are stimulated by male hormones produced by the adrenal glands of both males and females. Acne is not dangerous, but can leave scars on the skin. Human skin has pores (tiny holes) which connect to oil glands located under the skin. The glands are connected to the pores via follicles or small canals. These glands produce Sebum, an oily liquid which carries dead skin cells through the follicles to the surface of the skin. Pimples happen when these follicles get blocked, resulting in an accumulation of oil under the skin.In humans, pimples appear on the face, back, chest, shoulders and neck. The skin cells, sebum and hair can clump together into a plug, getting infected with bacteria, resulting in a swelling. A pimple starts to develop when the plug begins to break down. The defence mechanism of body acts to kill the bacteria, moulding in formation of whiteheads, blackheads, and pustules in these areas.

1.2 TYPES OF ACNE/PIMPLES

a. Whiteheads: These remain under the skin and are very small.

b. Blackheads: These are clearly visible, are black in colour and appear on the surface of the skin. It is not caused by dirt. So scrubbing face vigorously will not help.

c. Papules: These are visible on the surface of the skin. These are small bumps, usually pink.

d. Pustules: They are clearly visible on the surface of the skin. They are red at their base and have pus at the top.

e. Nobules: These are large solid pimples clearly visible on the surface of the skin. They are painful and are embedded deep in the skin.

f. Cysts: These are painful, filled with pus and are clearly visible on the surface of the skin. It causes scars on the skin.

All these types of acne in general are termed as acne or pimples. Nobules and cysts are generally found in person with very oily skin and are severe in nature and takes time to heal.

1.3 CAUSES OF ACNE

a. Hormones: Common acne in teenagers starts with an increase in hormone production. During puberty, both boys and girls produce high levels of androgens, the male sex hormones that include testosterone. Testosterone signals the body to produce more sebum, the oil produced by oil glands of the skin.

b. Bacteria: Excess sebum clogs the openings of hair follicles on the face, neck, chest, and back allowing the bacteria to grow in these clogged follicles. This causes blackheads or whiteheads, also known as "comedones," to form on the surface of the skin. Sometimes, this clogging causes the follicle wall to break under the pressure of this buildup causing the sebum to leak into nearby tissues and forms a pustule or a papule which is inflammatory acne. Larger, tender pustules are called nobules.

c. Diet: Consumption of food having high glycemic index, dairy products, spicy and oily food products exaggerates sebaceous glands activity leading to acne. Smoking and alcohol consumption also leads to acne.

d. Cosmetics: Excessive use of cosmetic products or silicon containing products clogs the pore leading to formation of whiteheads. Dead skin cells on the skin surface or dirt forms blackheads when mixed with sebum. Scrubbing face vigorously or pricking pimple at this time worsens the situation leading to scar formation.

e. Drugs: Prolonged use of some drugs burn the skin of that area causing scar formation. The oral contraceptives, injectable contraceptives, intrauterine birth control devices (IUD), Steroids taken by bodybuilders and athletes may also cause acne. This abnormal sebum, changes the activity of an usually harmless skin bacterium known as P. acnes, which becomes more aggressive and causes inflammation and formation of pus. Though acne cannot be fully cured, but can be controlled with proper treatment.

2. MATERIAL AND METHOD

2.1 Selection of plant material

The plants have been selected on its availability and folk use of the plant.

2.2Collection of plant material

Every part of the plant may contain active secondary metabolites, such as bark, leaves, flowers, roots, fruits and seeds. Fresh & healthy, disease free plant leaves of *L. Indica* were collected from ruler area of Raisen (M.P.) in the month of September, 2018and identified by Dr. Jasvinder Mehta, Department of Botany, Career College, Bhopal (M.P.).

2.3 Percentage loss (Harborne *et al.,* 1973)

The weight of the fresh sample and dried powder was determined, and the percentage loss was calculated due to drying and water loss.

The percentage loss was calculated by using following formula:

$$\%\ \textbf{Loss of drying} = \frac{Loss\ in\ weight\ of\ the\ sample}{Weight\ of\ the\ sample} x\ 100$$

2.4 Determination of ash values:

3 g of precisely weighed dried powder leaves were leaves of *L. Indica* dried powder

incinerated, cooled and weighed in a tarred platinum or silica dish at a temperature not exceeding 450°C until they were free of carbon. If this could not produce a carbon-free ash, the burnt mass was drained with hot water; the dust was deposited on the ah less filter paper, incinerated along with filter paper, evaporated to dryness and burned at a temperature not exceeding 450°C.

$$\textbf{Ash Value} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \text{ x } 100$$

2.5 Extraction procedure

Extraction from plant materials is an important step in phytochemical processing for discovering bioactive secondary metabolite. Selection of a suitable extraction technique is also important for the standardization of herbal products. Extraction is used to remove desirable soluble constituents, with the help of the selected solvents excluding those not required. At room temperature, all materials were dried out in shade. In a maceration method, the shade dried plant material was heavily powdered and subjected to extraction with petroleum ether (60-80 °C). The extraction was carried on until the material had been defatted. 65 gram of *Lagerstroemia Indica* was exhaustively extracted with different solvent (ethanol, aqueous) by maceration method. Over their boiling points the extract was evaporated. Finally, the percentage yields for the dried extracts are determined (Pradhan *et al.*, 2010; Ansari, 2001; Mukherjee, 2007).

2.6 Determination of percentage yield

The extraction yield is an evaluation of the solvent's efficiency in extracting bioactive components from the selected natural plant samples. Following formula was adopted for determination of percentage yield of selected plant materials. The percentage yield of each extract was calculated by using following formula:

$$\textbf{Percentage Yield} = \frac{\text{Weight of Extract}}{\text{Weight of Powder drug taken}} \text{ x } 100$$

2.7 Phytochemical screening

Medicinal plants are traditional medicinal resources and many of the modern medicinal products are produced indirectly from plants. Phytochemical components consist of two primary bioactive components (chlorophyll, proteins, amino acids, sugar, etc.) and secondary bioactive components (alkaloids, terpenoids , flavonoids, etc.). Phytochemical examinations were carried out for all the extracts as per the standard methods.

2.8 Quantitative estimation of bioactive compounds

2.8.1 Total phenolic content estimation

The extract's total phenolic content was measured using the modified Folin-Ciocalteu method. 10 mg of gallic acid was dissolved in 10 ml of methanol, and a number of 10-50µg / ml aliquots were prepared in methanol.10 mg of dried extract was dissolved in 10 ml of methanol. 2 ml (1mg / ml) of this extract had been used for phenol calculation.2 ml of extract and 1 ml of Folin-Ciocalteu reagent (previously diluted with 1:10 v / v distilled water) and 1 ml (7.5 g / l) of sodium carbonate were mixed each standard. The mixture was vortexed for 15s and allowed color production to stand for 10min. The absorbance measurement with spectrophotometer at 765 nm. (Olufunmiso*et al.*, 2011).

2.8.2 Total flavonoids content estimation

The method used to determine the total content of flavonoids was using aluminum chloride. In 10 ml of methanol, 10 mg of quercetin was dissolved and various 10-50µg / ml aliquots were prepared in methanol. 10 mg of the dried extract was dissolved in 10 ml of methanol and filter. Three ml (1mg / ml) of this extract and 1 ml aluminum chloride was used for calculating flavonoids. (Olufunmiso *et al.*, 2011).

2.9 Antioxidant activity of extracts by DPPH scavenging method

DPPH scavenging activity was assessed with spectrophotometer. Storage solution (6 mg in 100 ml methanol) was prepared to give 1.5 ml of it in 1.5 ml of methanol as an initial absorption. Decline in absorbance was observed at different concentrations (10-100 µg / ml) after 15 minutes when sample extract was present. 1.5 ml of DPPH solution was taken and

methanol volume was produced up to 3 ml, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally the mean was taken. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm (Tiwari et al., 2017).

$$\% \text{ Reduction} = \frac{\text{Control Absorbance} - \text{Test absorbance}}{\text{Control Absorbance}} x100$$

2.10 Antimicrobial and anti-acne activity of extracts

Method of preparation

This agar medium has been dissolved in distilled water and boiled with adequate capacity in conical flask. The dry ingredients are transferred to a flask containing the required amount of distilled water and heat to completely dissolve the medium (Bauer *et al.*, 1996). The flask containing medium was cotton plugged and was placed in autoclave for sterilization at 15 lbs /inch^2 (121^{o}C) for 15 minutes. The media in flask (20 ml / plate) was automatically poured onto sterile petri dishes on the plane surface upon sterilization. The poured plates were left overnight at room temperature to solidify and incubate at 37^{o}C to test plate sterility. During 30 minutes prior to using the plates were dried at 50^{o}C. The lyophilized form was obtained from the microbial cultures used in the analysis. The lyophilized colonies are inoculated in sterile nutrient broth with the aid of aseptic techniques then they are incubated at 37^{o}C for 24 h. In turbidity development is observed after incubation. These broth cultures were further inoculated on to the nutrient agar plates with loop full of microbes and further incubated for next 24 hours at 37^{o}C to obtain the pure culture and stored as stocks that are to be used in further research work.

2.11 Antiacne sensitivity

The antiacne sensitivity test is employed on to the all selected microbes used under present study with different extracts obtained from leaves of *L. Indica*. For this experiment 6 mm diameter wells, stock of 25 mg/ml, 50 mg/ml, 100 mg/ml of extract separately applied on it. A nutrient agar plate is seeded with particular microbes with the help of spread plate technique prior and left for 5 minutes then incubated for 24 hours at 37 ^{o}C. After incubation, plates were observed to see the sensitivity of extracts towards test bacteria of P.acne at particular concentration in the form zone of inhibition.

2.12 Antibiogram Studies

The well diffusion method was used to determine the antiacne activity of extracts prepared from the leaves of *L. Indica* using standard procedure (Bauer, 1966). In antibiogram studies, 3 concentrations were used that are 25, 50 and 100 mg/ml for every extracted phytochemicals. It's essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted over night broth cultures should never be used as an inoculums. During 24 hours the plates were incubated at 37^{o}C and then examined for clear inhibition zones around the wells impregnated with a particular concentration of drug.

3. RESULT AND DISCUSSION

The dried Leaves material was washed carefully under running tap water and was grinded using electronic grinder. The powder was extracted by maceration method, using solvent ethanol and aqueous. *L. Indica* was tested on the different standardization criteria such as organoleptic measurement, percentage loss, percentage yield, and phytochemical screening.

3.1 Results of percentage loss

Table 3.1: Showing the results of percentage loss of *L. Indica*

S. No.	Plant name	Description	Weight in (gms.)	% loss
1.	*L. Indica*	Weight of plant material (Leaves) in wet, fresh condition	100	**55%**
2.		Weight of plant material (Leaves) after drying at room temperature	45	
3.		Loss in weight on drying	100-45=55	

3.2 Results of Physio-chemical parameters of plants powder

Table 3.2: Results of percentage ash value of leaves of *L. Indica*

S. No.	Parameters	*L. Indica*
1.	Total ash value	1.7%

3.3 Results of percentage yield

Table 3.3: Results of percentage yield of leaf extracts of *L. Indica*

Plant Name	Percentage yield (%)	
	Ethanol	**Water**
L. Indica	9.52	8.38

Table no. 3.3 showed the percentage yield of different extract of leaves of *L. Indica* exhibited higher yield in ethanolic extract and 9.52% respectively. Aqueous extract of leaves of *L. Indica a*lso exhibited comparable yield 8.38%.

3.4 Results of phytochemical screening of extracts

Preliminary phytochemical analysis generally helps identify and classify the plant extracts' bioactive constituents. For extracts of all samples, a small portion of the dried extracts of plant leaves underwent phytochemical screening using Kokate (1994) methods for chemical testing of alkaloids, glycosides, flavonoids, saponins, phenolics, proteins and amino acids, diterpenes and tannins separately.

Table 3.4: Result of Phytochemical Screening of extracts of *Lagerstroemia Indica*

S. No.	Constituents	Ethanol extract	Aqueous extract
1.	Alkaloids		
	A) Wagner's Test:	-Ve	-Ve
	B) Hager's Test:	+Ve	-Ve
2.	Glycosides		
	A) Legal's Test:	+Ve	+Ve
3.	Flavonoids		
	A) Lead acetate Test:	+Ve	+Ve
	B) Alkaline Reagent Test:	+Ve	+Ve
4.	Saponins		
	A) Froth Test:	+Ve	-Ve
5.	Phenolics		
	A) Ferric Chloride Test:	+Ve	+Ve
6.	Proteins and Amino Acids		
	A) Xanthoproteic Test:	-Ve	-Ve
7.	Carbohydrate		
	A) Fehling's Test:	+Ve	-Ve

8.	Diterpenes A) Copper acetate Test:	-Ve	-Ve
9.	Tannin A) Gelatin test:	+Ve	+Ve

Flavonoid was detected in different extracted leaves of *Lagerstroemia Indica*. The *Lagerstroemia Indica* leaves, ethanolic and aqueous extract showed presence of Phenols and Flavonoids. The ethanolic extracts possess almost all the phytochemicals that were tested when compared other solvent. It could be seen from table no. 3.4 that glycosides, flavonoids, saponins, tannins, alkaloids and phenolics were present in the Lagerstroemia *Indica* ethanol extract of *Lagerstroemia Indica*.

3.5 Results of estimation of total phenol and flavonoids content

Table 3.5: Estimation of total phenolic and flavonoids content of *Lagerstroemia Indica*

S. No.	Extracts	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1.	**Ethanol**	4.957	3.875
2.	**Aqueous**	5.316	4.537

The presence of phytochemicals (Phenols and Flavonoids) was quantitatively screened. Table No. 3.5 shows the overall flavonoid content of the Lagerstroemia *Indica* of ethanol and aqueous extract. The total flavonoid content of ethanol and aqueous (equivalent to quercetin) was 3.875, and 4.537mg/100 mg respectively In *Lagerstroemia Indica*, the quantitative analysis of the total phenolic content showed that aqueous extract was the richest source of phenols than ethanolic extract. The ethanol and aqueous extract quantitative analysis revealed total phenolic content (equivalent to gallic acid) of 4.957mg/100 mg and 5.316 mg/100 mg respectively.

3.6 Results of Antioxidant activity of leaves extracts of *L. Indica*

DPPH (1, 1-Diphenyl-2-picrylhydrazyl radical) reduction was investigated against normal ascorbic acid for evaluating antioxidant function of selected plant extracts. The amount of DPPH decolonisation was attributed to the ability of plant extracts to contribute hydrogen. Free radicals cause many serious illnesses. Thus, antioxidant activity evaluation in selected plants can contribute to the discovery of natural antioxidants. The present research should also be used in pharmaceutical applications to test experimental medications. A comparative study of antioxidant activity of three medicinal plants *L. Indica* were carried out in this study. Scavenging of stable radical (DPPH radical) was recorded *in vitro* IC_{50} was calculated as amount of antioxidant present in the plant extract.Thus, the present study aim was to assess the antioxidant activity of *L. Indica* by DPPH method and also compared the % antioxidant activity with standard ascorbic acid.

3.6.1 Results of Antioxidant activity using DPPH method

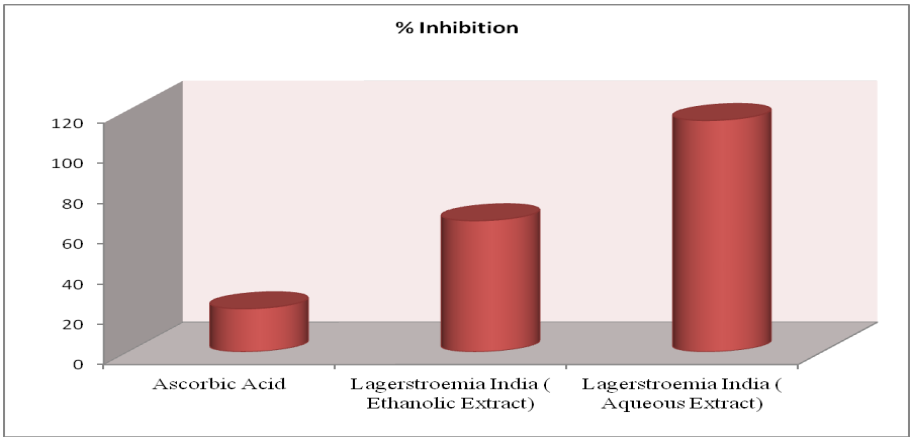

Figure 3.1: Graph of IC_{50} value of standard and plant extract using DPPH method

The concentration of ethanol and aqueous extract of different parts of each medicinal plant taken to inhibit 50% of DPPH was indicated.Variable DPPH activity was recorded for ethanolic extract of *Lagerstroemia Indica* and aqueous extract of *Lagerstroemia Indica.* The DPPH scavenging activity of ethanolic extract of *Lagerstroemia Indica* and aqueous extract of *Lagerstroemia Indica* were showed IC50 value 65.14µg/ml and 114.96µg/ml as compared to positive control ascorbic acid 21.41µg/ml. The aqueous extract of *Lagerstroemia Indica* showed less antioxidant activity (IC50 114.96µg/ml) because it contains lesser amount of phenols and flavonoids.

3.7 Results of Anti Acne activity

The antimicrobial activity of plant material of LIE (*Lagerstroemia Indica ethanolic* extract), LIA (*Lagerstroemia Indica* aqueous extract), were evaluated against bacteria(*Propionibacterium acnes*) under present study. The fresh pure 100% extracts obtained from selected plants used to suitably dilute up to the concentrations of 100, 50 and 25mg/ml and applied on to the *Propionibacterium acnes* using agar well diffusion method. Results of the experiment were being concluded in which clearly showed the anti- acne activity of extracts of LIE (*Lagerstroemia Indica ethanolic* extract), LIA (*Lagerstroemia Indica* aqueous extract) against *Propionibacterium acnes* strains.

Table 3.6:Anti-Acne activity of extracts against *Propionibacterium acnes*

Microbes→ Extract↓	Propionibacterium acnes		
	100 mg/ml	**50 mg/ml**	**25mg/ml**
LIE Extract	22±9.14	19±8.9	16±9.16
LIA Extract	16±1.86	13±2.11	8±1.21
Clindamycin	26±2.85	21±3.28	18±1.72

A cursory look at table no. 3.6 showed Anti-acne activity of extracts against *Propionibacterium acnes* that *Lagerstroemia Indica* ethanol extract showed greater capacity against *Propionibacterium acnes* (16±9.16, 19±8.9, 22±9.14) to inhibit bacterial growth. Results obtained from the study indicated that the plant extract showed the strongest anti acne activity.

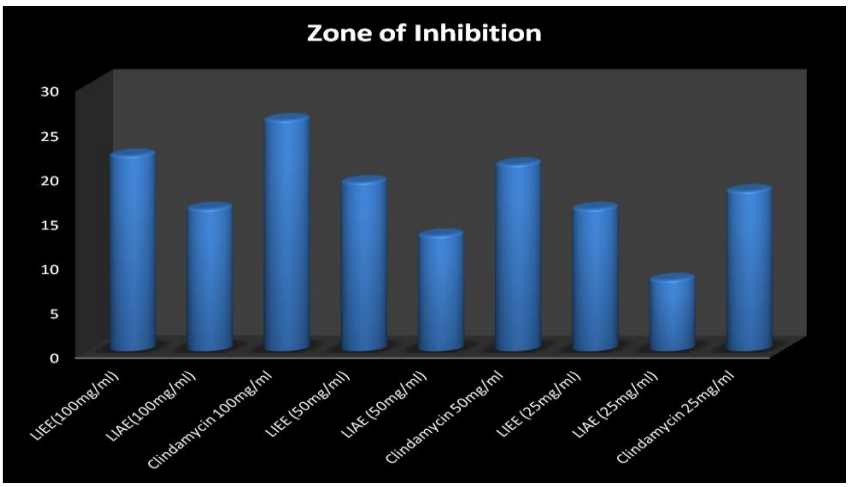

Figure: 3.2 Anti-Acne activity of extracts against *Propionibacterium acnes*

Discussion

Acne vulgaris is a chronic inflammatory disease results in the formation of inflamed and/or non-inflamed eruptions Propionibacterium acnes are the anaerobes, in the skin which grow in the sebaceous region. Various antibiotics like tetracycline, Clindamycin, and erythromycin etc and other drugs like benzoyl peroxide are used for acne treatment. The various drawbacks of synthetic drugs are different side effects and resistant developed towards these drugs. Herbal therapy is required to overcome the above

drawbacks and treat the acne. So in the present study plants (*Lagerstroemia Indica*) were selected for the anti acne activity. The preliminary phytochemical study was carried out according to standard literature. This revealed that they contain various phytoconstituents which can be responsible for the anti acne activity. The extracts were subjected to antimicrobial activity against Propionibacterium acnes. The MIC obtained from the various extracts against P. acnes suggests that the ethanolic extracts of the plants showed significant antimicrobial activity.

The acne like inflammatory activity was carried out by measuring the ear thickness and histopathological studies of the ear. Ethanolic extracts of the plants showed significant reduction in the ear thickness. It seems that the increased ear thickness and inflammation caused due to various biochemicals, viz. various kinins, histamine and 5-HT is significantly reduced. *Lagerstroemia Indica ethanolic* extract are the active anti-inflammatory constituents. The anti-inflammatory activity of *Lagerstroemia Indica ethanolic* extract may be due to its ability to scavenge oxygen radicals which has been implicated in inflammation process. All the four plants have proved to possess anti-inflammatory activity. The ethanolic extracts of plants showed significant reduction in the over all damage caused due to P. acne which can be seen in the histopathological studies. The literature review shows that inflammation is caused due to ROS (Reactive Oxygen Species). The ethanolic extracts of *Lagerstroemia Indica contain* alkaloids, flavanoids etc. The above phytoconstituents were proved potent anti-oxidants. The presence of various phytoconstituents including the flavanoids, Tannin, alkaloids in *Lagerstroemia Indica ethanolic* extract showed significant anti-acne properties which is supported by the antimicrobial and histopathological studies.

Conclusion

Acne vulgaris is a common skin affliction affecting millions of lives. There are plenty of exceptional care options to consider for acne cure. Study showed that our traditional context masks a range of amazing herbs that could be healthy and effective trade to pimples for care. Pharmaceuticals are searching for a viable solution to come free from disorders ranging from unsightly blemishes and disfiguring inflammation attributable and acne for dedicated teenagers and diverse communities. The use of innovative technology based on such herbs as successful cutting-edge pores and skin care components needs to be included in continuous and non-stop testing. This research work is intended to inspire and motivate valuable tool practitioners, cosmetics experts, scientists, pharmacists, industrialists and dermatologists to use these herbs more specifically in the topical dermato-cosmetic system so that customers can benefit more from natural substances. From the long list of herbs in my research *L. Indica* are some of the common herbs that are found in abundant. The chosen plants develop an effectivel Anti-acne active.Numerous drugs for the treatment of skin inflammation vulgaris are available in the commercial market. Many of those meanings are anti-microbial topical agents and so on. In either case, there are multiple signs of these plans. P. acnes induces antimicrobial resistance and thereby prevents the application of anti-microbials. It is anything but difficult to take out such symptoms with home grown topical plants of *L.* active. Consequently utilization of natural definitions is more helpful to treat skin break out vulgaris.

Reference

1. Levy SB, Marshall B. Antibacterial resistance worldwide: causes, challenges and responses. Nature Medicine Supplement. 2007; 10(12):122-129.
2. Walsh FM, Amyes SGB. Microbiology and drug resistance mechanisms of fully resistant pathogens. Current Opinion in Microbiology. 2004; 7:439–444.
3. Paul R, Prasad M, Sah NK. Anticancer biology of *Azadirachta indica* L (neem): A mini review. Cancer Biology and Therapy. 2011; 12(6):467-476.
4. Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Reviews. 1999; 12:564–582.

5. Avancini CAM, Wiest JM, Mundstock EA. Bacteriostatic and bactericidal activity of the Baccharis trimera (Less.) D.C. Compositae decocto, as disinfectant or antisseptic. Arq Bras Med Vet Zootec. 2000;52(3):230-4.
6. Alzoreky NS, Nakahara K. Antibacterial activity of extracts from some edible plants commonly consumed in Asia. Int J Food Microbiol. 2003; 80(3):223-30.
7. Machado TB, Pinto AV, Pinto MC, Leal ICR, Silva MG, Amaral ACF, et al. In vitro activity of Brazilian medicinal plants, naturally occurring naphthoquinones and their analogues, against methicillin-resistant Staphylococcus aureus. Int J Antimicrob Agents. 2003;21(3):279-84.
8. Candan F, Unlu M, Tepe B, Daferera D, Polissiou M, Sokmen A, et al. Antioxidant and antimicrobial activity of the essential oil and methanol extracts of Achillea millefolium subsp. millefolium Afan. (Asteraceae). J Ethnopharmacol. 2003; 87,2- 3.
9. Yap PSX, Yiap BC, Ping HC, Lim SHE. Essential oils, A new horizon in combating bacterial antibiotic resistance. The Open Microbiology Journal. 2014; 8:6-14**.**
10. Fadli M, Saada A, Sayadi S, Chevalier J, Mezrioui NE,Pagès JM, Hassani L. Antibacterial activity of Thymus maroccanusand Thymus broussonetiiessential oils against nosocomial infection – bacteria and their synergistic potential with antibiotics. Phytomedicine. 2012; 19:464–471.
11. Gallucci N, Casero C, Oliva M, Zygadlo J, Demo M. Interaction between terpenes and penicillin on bacterial strains resistant to beta-lactam antibiotics. Mol Med Chem. 2006; 10(1):30-238.
12. Hartmann, T. (2008). The lost origin of chemical ecology in the late 19th century. Proceedings of the National Academy of Sciences of the United States of America, Vol. 105, No. 12, (March 2008), pp. 4541-4546.
13. Flaynn CL, Bronaugh RL and Maibach HI, Percutaneous absorption, Marcel Dekker,New York, 1985,17-52.
14. Kasting CB, Smith RL, Andersion BD, Topical and ocular drug delivery, Marcel Dekker, New York,1992,117-61.
15. Potts RD, Guy RH, Predicting skin permeability, Pharm Res, 1992, 9,663-9.
16. Scheuplein RJ, Bank IH, Permeability of the skin, Physiol Rev, 1971, 51,702-47.
17. Scheuplein RJ, Bank IH, Brauner CJ, Macfalrane DJ, Precutaneous absoption of steroids, J Invest Dermatol, 1969, 52,63-70.
18. Menon CK. New insights into skin structure: scratching the surface, Adv Drug Deliv Rev, 2002, 54, 3-17.
19. Kaur D, Prasad SB. Anti acne activity of acetone extract of *Plumbago indica* root. Asian J Pharm Clin Res. 2016; 9(2):285-7.
20. Gupta MA, Gupta AK. Depression and suicidal ideation in dermatology patients with acne, alopecia areata, atopic dermatitis and psoriasis. Br J Dermatol 1998; 139(5):846-50.
21. Cunliffe WJ. Natural history of acne. In: Cunliffe WJ, editor. Acne. London: Martin Dunitz; 1989; 2-10.
22. Date AA, Naik B, Nagarsenker MS. Novel drug delivery systems: potential in improving topical delivery of antiacne agents. Skin Pharmacol Physiol 19(1):216 (2006).
23. Schafer-Korting M, Korting HC, PoncePoschl E. Liposomal tretinoin for uncomplicated acne vulgaris. Clin Investig 72(12):1086-91 (1994 Dec).
24. Brisaert M, Grabriels M, Matthijs V, et al. Liposomes with tretinoin: a physical and chemical evaluation. J Pharm Biomed Anal 26(5-6):909-17 (2001 Dec).
25. Rouse JG, Yang J, Ryman-Rasmussen JP, et al. Effects of mechanical flexion on the penetration of fullerene amino acidderivatized peptide nanoparticles through skin. Nano Lett 7(1):155-60 (2007 Jan).
26. Huczko A, Lange H. Fullerenes: experimental evidence for a null risk of

skin irritation and allergy. Fullerene Sci Technol 7:935-9 (1999).
27. Ansel H.C., Allen L.V., "Pharmaceutical Dosage Forms and Drug Delivery System", 7th edition, Lippincott Willams and Wilkens, Baltimore, 2000, 244246, 249-251, 253-255,264-265.
28. Nayank S.H., Nkhat P.D., and Yeole P.G., "The Indian Pharmacist", Vol. III, No. 27, Sept. 2004, 7-14.
29. Uche DOV, Sol-gel technique: A veritable tool for crystal growth, Advances in applied science research, 4(1), 2013, 506-510.
30. Carter SJ, Disperse system In: Cooper and Gunn's Tutorial Pharmacy, 6, CBS Publishers and Distributors, New Delhi, 2000, 68-72.
31. Lieberman HA, Rieger MM, Banker GS, Pharmaceutical dosage form: Disperse system, 2, Marcel Dekker, New York, 2005, 399-421.
32. Singh M. K., Khare G., Iyer S., Sharma G., Tripathi D. K. *Clerodendrum serratum*: A Clinical approach. JAPS. 2012: 2(2): 11-15.
33. Safwan Ali Khan M. Khatoon N. Al-Sanea M.M. Gamal Mahmoud M. Rahman H.U. Methanolic extract of leathery murdah, *Terminalia coriacea* (Roxb.) wight and arn. leaves exhibits anti-inflammatory activity in acute and chronic models. Med Princ Pract 2018; 27:267–271.
34. Vishwajeet Singh, Alok Bharadwaj, Nitin Wahi and Vijay Laxmi Tripathi. Phytochemical analysis and *in vitro* antimicrobial activities of *Terminalia arjuna* leaf, bark and fruit extracts in different solvents. Current Trends in Biotechnology and Pharmacy. 2018; 12(3): 286-294.
35. Sharma R, Raizada S, Gautam A, Bhatia AK. Phytochemical and antibacterial analysis of *Terminalia chebula* and *Terminalia bellirica*. In Green Chemistry in Environmental Sustainability and Chemical Education. 2018; 131-137.
36. Balaji Meriga, Parim Brahma Naidu, Ganjayi Muniswamy, GEN Hanuma Kumar, Ramavat Ravindar Naik and Suresh Pothani. Ethanolic Fraction of *Terminalia Tomentosa* Attenuates Biochemical and Physiological Derangements in Diet Induced Obese Rat Model by Regulating Key Lipid Metabolizing Enzymes and Adipokines. Pharmacogn Mag. 2017; 13(51): 385–92.
37.