IJPSR (2017), Vol. 8, Issue 1 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 07 July, 2016; received in revised form, 07 November, 2016; accepted, 09 November, 2016; published 01 January, 2017

PHARMACOLOGICAL AND ANTIBACTERIAL ASPECT OF *PSIDIUM GUAJAVA* L. AGAINST ACNE VULGARIS

Manisha Pandey, Afifa Qidwai, Rajesh Kumar, Anand Pandey, Shashi Kant Shukla, Ashutosh Pathak and Anupam Dikshit *

Biological Product Laboratory, Department of Botany, University of Allahabad, Allahabad-211002, Uttar Pradesh, India.

Keywords:

Pleomorphic, Comedones, Pustules, Antibacterial, CLSI, Tetracycline

Correspondence to Author:
Anupam Dikshit

Biological Product Laboratory, Department of Botany, University of Allahabad, Allahabad - 211002, Uttar Pradesh, India.

E-mail: anupambplau@rediffmail.com

ABSTRACT: Acne is a cutaneous pleomorphic disorder of the pilosebaceous unit involving abnormalities in sebum production and is characterized by both inflammatory (papules, pustules and nodules) and noninflammatory (comedones, open and closed) lesions. A lot of factor *viz;* hormone, increased sebum production and diet causes Acne vulgaris but bacteria are the chief causal organism in which *Propionibacterium* acnes and Staphylococcus epidermidis are considered as the major skin bacteria that cause the formation of acne. The present study was conducted to evaluate the *in vitro* antimicrobial activities of *Psidium guajava* L. leaves essential oil against *Propionibacterium* acnes and *Staphylococcus epidermidis*. Extraction of essential oil was carried out using Clevenger's Apparatus. Antibacterial activities of essential oil of leaves were investigated using broth micro dilution methods recommended by CLSI. The results showed that Guava leaves oils more effectively inhibit the growth of *P.acnes* (MIC: 0.321, IC50:0.309 mg/ml) as compared to *S. epidermidis* (MIC: 0.486, IC50:0.416 mg/ml). The readings were compared by standard drug tetracycline.

INTRODUCTION: Acne is one of the most common skin conditions which affect about 85 % of population some point of time in life [1]. It affects the areas of the body that have high concentrations of sebaceous gland such as the face, back and trunk [2]. The pathogenesis of Acne vulgaris is multifactorial, including increased sebum production, comedogenesis, proliferation of bacteria and inflammation [3]. The normal bacterial flora of the skin includes *Propionibacterium* acnes, *Staphylococcus epidermidis* and *S. aureus* which proliferate during puberty and often are involved in the development of acne [4].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.8(1).145-50

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8 (1).145-50

P. acnes, anaerobic bacteria play an important role not only in the process of inflammation but also in the formation of comedones while *S. epidermidis* and *S. aureus, aerobic bacteria* that usually involved in superficial infections of the sebaceous gland [5]. Anaerobic bacteria contribute to the inflammatory nature of acne by inducing monocytes to secrete pro-inflammatory cytokines including interleukin (IL)-1b, IL-8, and tumor necrosis factor (TNF)-a [6].

It is pronounced in puberty during adolescence and is positively related to sebaceous gland function, particularly in teenagers [7, 8], which androgenically stimulate higher sebum secretion [9]. The secreted sebum normally contains a mixture of lipids, squalene, wax and cholesterol both in free and in ester forms and triglycerides that naturally provide a skin barrier function [10].

However, the resultant abnormalities in sebaceous gland because of hormonal effects alter sebum composition and linoleic acid content [11].

Thus, the skin barrier is impaired and colonization of normal flora is promoted. For many years antibiotics have been used for the treatment of acne. However, resistance to antibiotics has increased and in a multifactorial manner, which includes the bacteria-antibiotic relationship, the type of antibacterial and the characteristics of the host, among others. To overcome the problem of resistance to antibiotics, medicinal plants have been studied extensively as alternative treatments [12]. Natural treatment is cheap, claimed to be safe and having less side effect [13, 14]. It is also suitable raw material for production of new synthetic agents.

Psidium guajava L. (Guava), belonging to family Myrtaceae is a traditionally used plant because of its nutrition value and anti-inflammatory property [15]. It is rich source of Vitamin C and antioxidants which is beneficial for skin. Various parts of the plant are found to possess many pharmacological properties in which leaves contain essential oil with the main components being alfa pinene, beta-pinene, limonene, menthol [16, 17], flavonoids and saponins combined with oleanolic acid [18] helpful in treatment of acne. In present study antibacterial property of *P.guajava* L. leaves oil is used against treatment of both anaerobic and aerobic bacteria viz *P.acnes* and *S.epidermidis*.

MATERIALS AND METHODS:

Test Pathogens: A culture of the test organisms, *Propionibacterium acnes* (MTCC 1951) and *Staphylococcus epidermidis* (MTCC 435) were procured from Microbial Type Culture Collection, Chandigarh, India and media was procured from Hi-Media. The culture of anaerobic bacteria was maintained on anaerobic blood agar medium supplemented with sheep blood. Anaerobic environment was given to the bacterial culture by the Anaxomate advance instrument after which the culture was incubated for 48 hours at 37°C in CO_2 incubator to provide optimum temperature for bacteria growth (**Fig. 1**). The culture of aerobic bacteria was maintained on Nutrient Agar and put in BOD incubator for their proper growth.

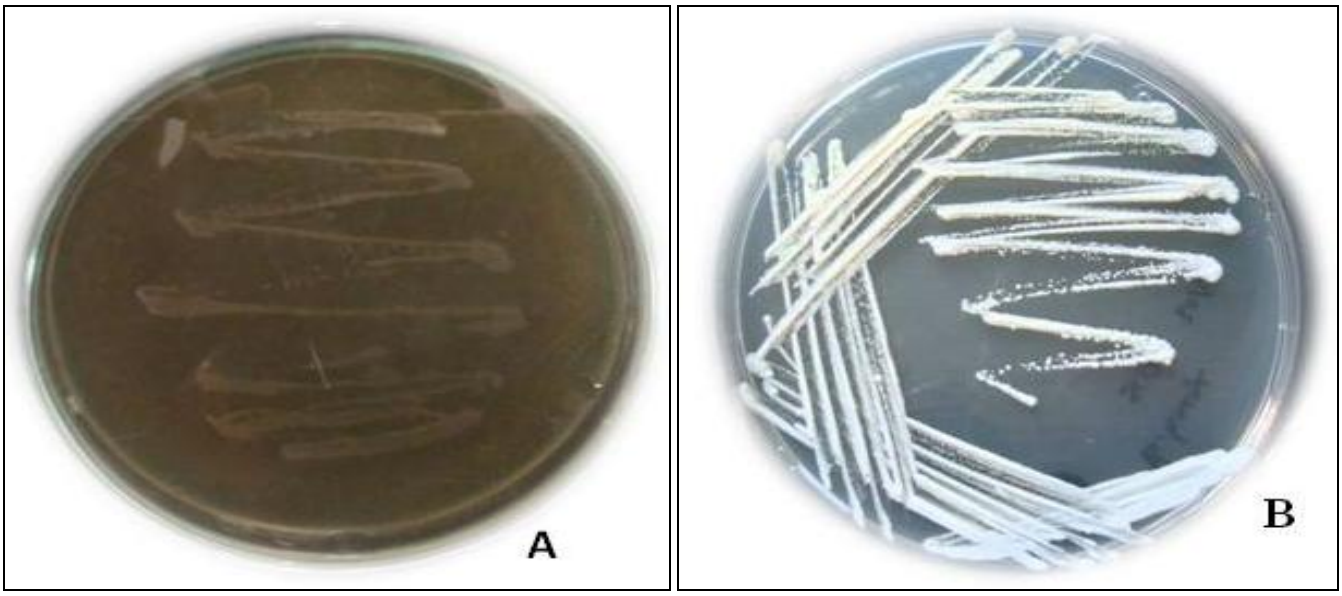

FIG. 1: A- CULTURE OF *P.ACNES* B- CULTURE OF *S.EPIDERMIDIS*

Extraction of Essential Oil: The leaves of *Psidium guajava* L. were collected from local area of Allahabad, washed thoroughly with tap water and then continuous flow of distilled water. Further, guava leaves were dried in shade to maintain their active constituents. Consequentially the leaves (200g) were cut into small pieces and placed into flasks (5L) with normal tap water (1.5L). Through steam distillation continuous extraction of oil was done using Clevenger's apparatus [19]. The water and oil mixture were separated by drying with anhydrous sodium sulphate (Na_2SO_4) and then filtered. The extracted oil was stored in refrigerator freezer at 4°C.

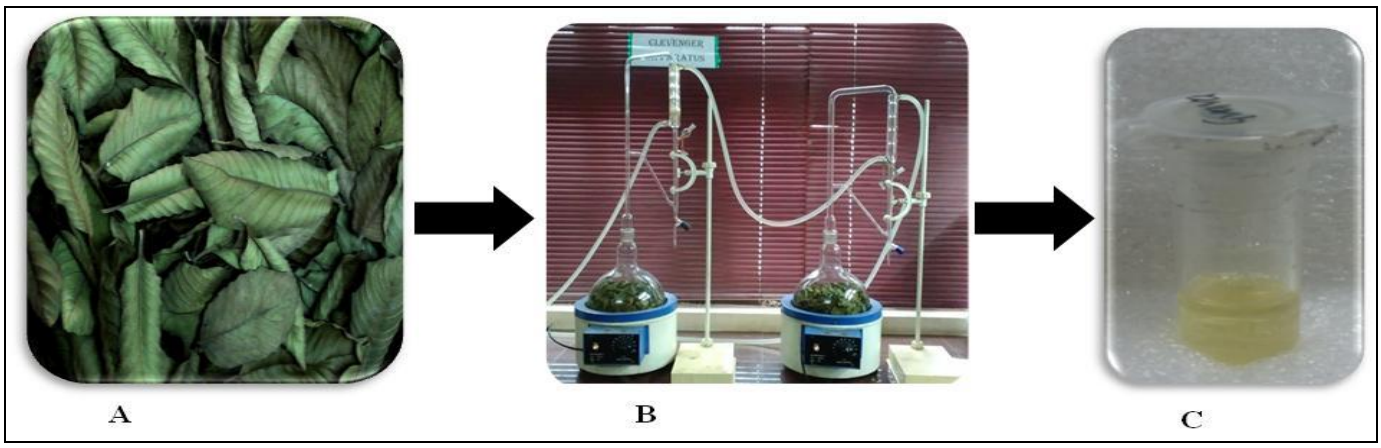

FIG. 2: A- DRIED LEAVES OF *P.GUAJAVA* L. B-CLEVENGER APPARATUS, USED FOR EXTRACTION OF OIL BY HYDRO DISTILLATION, C- *PSIDIUM GUAJAVA* L. LEAVES ESSENTIAL OIL

Antibacterial assay: The susceptibility of the *P. acnes* and *S. epidermidis* was assayed against *P. guajava* L. leaves oil using broth micro dilution method recommended by the Clinical and Laboratory Standards Institute (CLSI) [20]. Freshly prepared Muller Hinton Broth (MHB) medium was used for the assay. Stock solutions of essential oil of 50 mg/ml was prepared in 950 mg/ml of Dimethyl Sulfoxide (DMSO) and homogenized by using vortex for 4-5 min [21]. Bacterial inocula suspension was prepared as per 0.5 McFarland standards. Tetracycline, as a standard drug was also subjected to antibacterial assay. The experiment was performed according to CLSI guidelines in flat bottom sterile 96-well micro-titre plates, cultured overnight. Initial dispensing of 100 µl medium (MHB) was followed by the addition of 90 µl and 80 µl of MHB in columns 3 and 4 respectively. Further, 10 µl and 20 µl of drugs (*P. guajava* L. leaves essential oil) were added in each well of columns 3(sample control) and 4(dilution well) respectively. Following this, serial dilution was done from 4th column wells (2.5mg/ml) to 11th column wells (0.02mg/ml) and after dilution, was discarded from the last column.

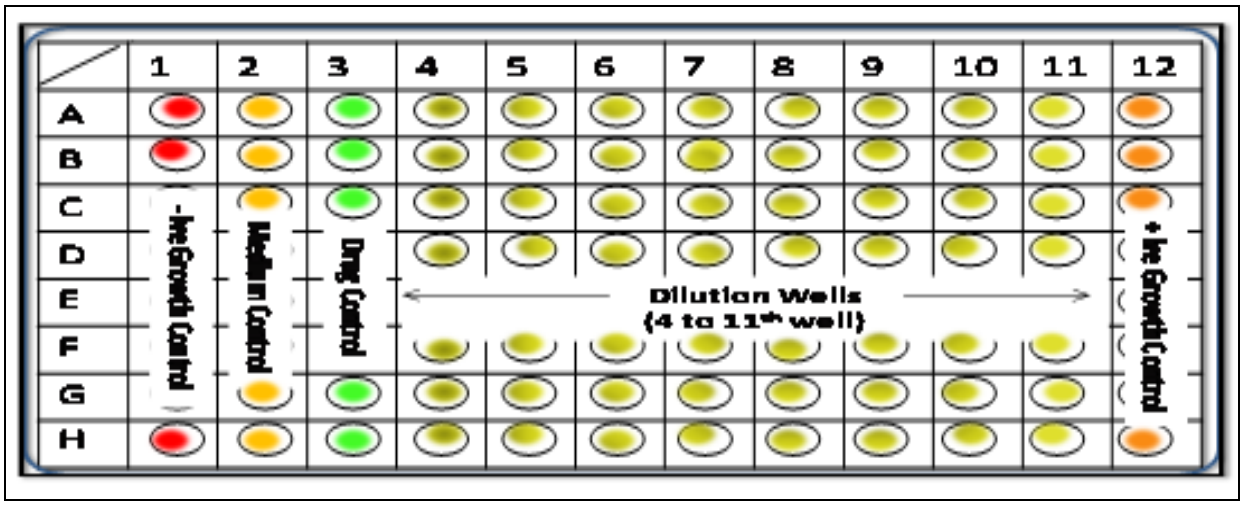

FIG. 3: PICTORIAL REPRESENTATION OF 96 WELL PLATES IN CLSI RECOMMENDED BROTH MICRO DILUTION TECHNIQUE

After the serial dilution, 100 µl of bacterial inoculum was added in each well of column 4 to column 12, so as to maintain final volume of 200 µl. Column 1 contained media and formaldehyde to serve as negative control. Column 12 was taken as the positive control (O. D. Control), which contains 100µl medium and 100µl inocula (**Fig. 3**). This experimented 96 well plate of anaerobic bacteria incubated in CO_2 incubator (Galaxy 170 S New Brunswick, USA) for 48 hours and aerobic bacterium 96 well plates were placed in BOD incubator for 24 hours.

Determination of Minimum Inhibitory Concentrations (MICs) and IC_{50}: The MICs and IC_{50} were obtained by measuring absorbance using spectrophotometer. For herbal oil, the MIC was determined as the lowest drug concentration showing absence of growth visually or 80% growth inhibition compared with the growth in the drug-free well. IC_{50} defined as the drug concentration that produces 50 % of growth inhibition compared to the growth in the drug-free well. Comparative inhibition percent of bacterium inoculum in media treated with herbal oil was calculated by using the following formula.

% Inhibition = (O.D. Control - O.D. treatment)/ O.D. control) × 100 [22].

RESULTS AND DISCUSSION: *Psidium guajava L. (Guava)* is a phytotherapic plant used in folk medicine that is believed to have active components that help to treat and manage various diseases [23, 24, 25]. The many parts of the plant have been used in traditional medicine to manage conditions like malaria, gastroenteritis, wounds, inflamed gums and a number of other conditions [26, 27].

In present study, antibacterial effects of the leaves of *P.guajava* L. essential oil were evaluated against *P.acnes* and *S. epidermidis* bacteria. The efficiency of antibacterial activity was quantitatively assessed with reference to the MICs as well as IC_{50} (mg/ml) values through 96 well micro-titer plate (CLSI recommended broth micro dilution method). Guava leaves essential oil were more effective against anaerobic bacteria (MIC: 0.321, IC_{50}:0.309) as compared to aerobic bacteria (MIC: 0.486, IC_{50}:0.416). This quantitative value were compared by control (tetracycline) reading against *P.acnes* (MIC: 0.028, IC_{50}: 0.013) and *S. epidermidis* (MIC: 0.159, IC_{50}:0.106) in **Table 1.**

TABLE 1: TABLE SHOWING MIC AND IC_{50} VALUE OF *P.GUAJAVA* L. LEAVES AND SYNTHETIC DRUG AGAINST *P.ACNES* AND *S.EPIDERMIDIS*

Testing against pathogen (Bacteria)	Drugs			
	P. guajava L.		Tetracycline (Control)	
	IC_{50} (mg/ml)	MIC (mg/ml)	IC_{50} (mg/ml)	MIC (mg/ml)
P. acnes	0.309	0.321	0.013	0.028
S. epidermidis	0.416	0.486	0.106	0.159

The whole plant of *Psidium guajava* L. consists of various pharmacological activity and rich in various active constituent such as tannin, flavonoids, phenols, triterpins, saponins, carotenoids, lectin, fibers, fatty acid, ascorbic acid, limonine, lysine, aspartic acid, polyphenols and terpins. Leaves of Guava is rich in flavonoids especially quercetin which is mainly responsible for antibacterial activity. It is also well known antioxidant, with antimicrobial properties [28].

Essential oils are natural composite mixtures of terpenic and non-terpenic compounds. Commonly monoterpenes and sesquiterpenes as well as their oxygenated derivatives are the major constituents. These are secondary metabolites which have antibacterial property [29]. Major compounds of essential oil of guava leaves are alpha-pinene (monoterpene). Alpha-pinene which is found in the oils of many species of coniferous trees, shows antimicrobial activity against wide range of bacteria and fungi [30] and it decreases sebum production by reducing the size of sebaceous glands thus controlling the spread of acne [31]. It might cross the cell membranes, thus penetrating into the interior of the cell and interacting with intracellular sites critical for antibacterial activity [32]. Additionally leaves contain L-ascorbic acid, i.e. main citrus acid that combat acnes.

The citric acid exfoliates the skin which is proved to be significant stage in treating acne. Using anti-inflammatory agents may also be helpful in reducing the inflammatory process in the acne lesions. *Psidium guajava* leaves were found to contain anti-inflammatory activity, by decreasing serum chemokines such as interleukin-8,eosinophil cationic protein [33] and having antioxidant activity [34].

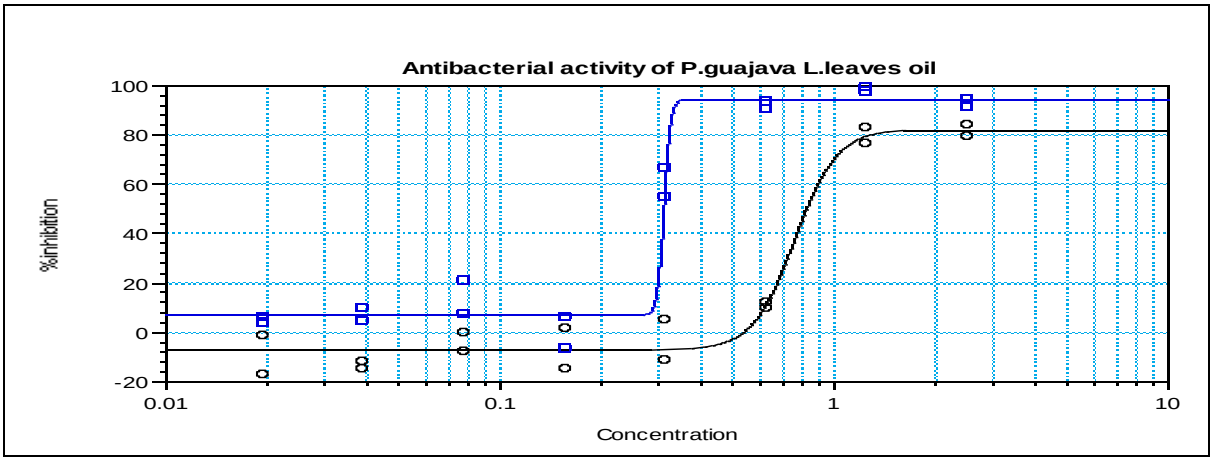

FIG. 4: GRAPH SHOWING ANTIBACTERIAL ACTIVITY OF *P. GUAJAVA* L.OIL (MIC AND IC_{50}) AGAINST *P. ACNES* (BLUE LINE) AND *S.EPIDERMIDIS* (BLACK LINE) AT 24 HOURS

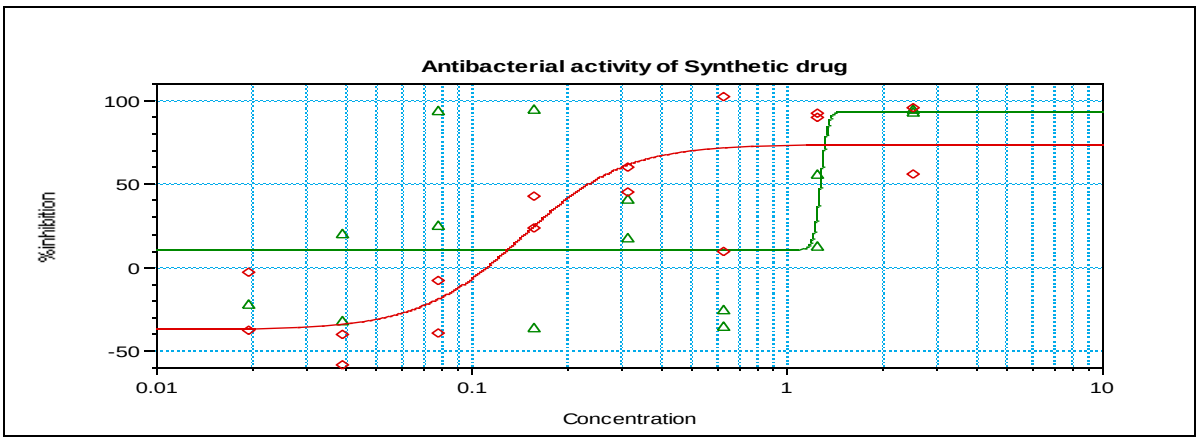

FIG. 5: GRAPH SHOWING ANTIBACTERIAL ACTIVITY OF STANDARD DRUG, TETRACYCLINE (MIC AND IC_{50}) AGAINST *P. ACNES* (GREEN LINE) AND *S. EPIDERMIDIS* (RED LINE) AT 24 HOURS

CONCLUSION: Although there are numerous drug therapies for the treatment of acne (topical, systemic retinoid, antibiotics, and keratolytics), the foremost challenge is the growing concerns of rising antibiotic resistance and dermal toxicities with existing medications. It is praise those natural remedies as an alternative against acne over these synthetic drugs. These developing natural therapies cover naturally derived drugs from active plant extracts, essential oils, and phytomolecules which are discussed in the above paragraph. Essential oil of *P. guajava* L. contains terpenoids, voletile oil, glycosides and Flavonoids; later one is the most important constituent responsible for anti-inflammatory activity beneficial in treatment of acne. Overall, herbal medicine has much to offer to improve our ability to deal with the complex issues of acne presents.

ACKNOWLEDGEMENTS: Thanks due to Head, Department of Botany, University of Allahabad to providing research facilities, UGC, New Delhi for financial support, Mr. Rick Z for providing anaerobic jar and Moti Lal Nehru Medical College, Allahabad for providing the anaerobic culturing facilities.

REFERENCES:

1. Bettoli V, Zauli S and Virgili A: Is hormonal treatment still an option in acne today. Br J Dermatol 2015; 1:37–46.
2. Leydon JJ: Therapy for acne vulgaris. The New England Journal of Medicine 1997; 336:1156-1162
3. Leyden J: A review of the use of combination therapies for the treatment of acne vulgaris. Journal of the American Academy of Dermatology 2003; 49(3):S200–210.
4. Hamnerius N: Acne-etiology and pathogenesis. Treatment of Acne 1996; 32: 29-38.
5. Burkhart CG, Burkhart C and Lehmann P F: Acne: a review of immunologic and microbiologic factors. Journal of postgraduate Medicine 1999; 75: 328-331.
6. Kim J: Review of the innate immune response in acne vulgaris: Activation of Toll-like receptor 2 in acne triggers inflammatory cytokine responses. Dermatology 2005; 211(3): 193–198.
7. Thiboutot D: Acne: hormonal concepts and therapy. Clin. Dermatol 2004; 22: 419–428.

8. White GM: Recent findings in the epidemiologic evidence, classification and subtypes of acne vulgaris. J. Am. Acad. Dermatol 1998; 39: 34–37.
9. Henderson CA, Taylor J and Cunliffe WJ: Sebum excretion rates in mothers and neomates. Br. J. Dermatol. 2000; 142: 110–111.
10. Pilgram GS, vander M J, Gooris GS et al.: The influence of two zones and sebaceous lipids on the lateral organization of lipid isolated from human stratum corneum. Biochim. Biophys. Acta 2001; 1511: 244–254.
11. Downing DT, Stewart ME, Wertz PW et al.: Essential fatty acids in acne. J. Am. Acad. Dermatol. 1986; 14: 221–225.
12. Bahmani M, Saki K, Rafieian-KM, Karamati SA, Eftekhari Z, Jelodari M: The most common herbal medicines affecting Sarcomastigophora branches: a review study. Asian Pac J Trop Med. 2014; 7S1:S14–21.
13. Rafieian-KM: Medicinal plants and the human needs. J Herb Med Pharmacol. 2013; 1(1):1–2. 9.
14. Singh R, Kaur N, Kishore L, Gupta GK. Management of diabetic complications: a chemical constituents based approach. J Ethno pharmacol. 2013; 150(1):51–70.
15. Aguilar A, Argueta A, Cano L: Flora Medicinal Ind´ıgena de M´exico. Instituto Nacional Indigenista, M´exico. 1994; 45(2)
16. Zakaria M, Mohd M A: Traditional Malay medicinal plants. Penerbit Fajar Bakti Sudan Berhard 1994; 129–132.
17. Li J, Chen F, Luo J: GC–MS analysis of essential oil from the leaves of *Psidium guajava*. Zhong Yao Cai 1999; 22, 78–80.
18. Arima H, Danno G: Isolation of antimicrobial compounds from guava (*Psidium guajava* L.). Bioscience, Biotechnology and Biochemistry 2002; 66, 727–1730.
19. Clevenger J F: Apparatus for the determination of volatile oil. J Am Pharm. Assoc.1928; 17: 346-346.
20. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically (5th Ed.), Approved standard, M7-A5. Pennsylvania: Wayne. NCCLS 2003.
21. Qidwai A, Pandey M, Shukla SK, Kumar R, Pandey A, Dikshit A: Antibacterial activity of *Mentha piperita* and citrus limetta against *Propionibacterium acnes* (anaerobic bacteria). International Journal of Pharmaceutical Sciences and Research 2016; 7(7): 2917-2924.
22. Pathak A, Shukla SK, Pandey A, Mishra RK, Kumar R, Dikshit A: In vitro antibacterial activity of Ethno medicinally used lichens against three wound infecting genera of Enterobacteriaceae. Proc. Natl. Acad. Sci. India B Biol. Sci. 2015; 1-2.
23. Abdelrahim SI, Almagboul AZ, Omer MEA, Elegami A: Antimicrobial activity of *Psidium guajava* L. Fitoterapia *2002*; 73:713–715.
24. Jaiarj P, Khoohaswan P, Wongkrajang Y, et al.: Anticough and antimicrobial activities of *Psidium guajava* Linn. leaf extract. Journal of Ethnopharmacology *1999;* 67(2):203–12.
25. Lutterodt GD: Inhibition of Microlax-induced experimental diarrhoea with narcotic-like extracts of *Psidium guajava* leaf in rats. Journal of Ethno pharmacology *1992; 37(2):151–157.*
26. Begum S, Hassan SI, Ali SN, Siddiqui BS: Chemical constituents from the leaves of *Psidium guajava*. Natural Product Research *2004; 18(2):135–140.*
27. Sunagawa M, Shimada S, Zhang Z, Oonishi A, Nakamura M, Kosugi T: Plasma insulin concentration was increased by long-term ingestion of guava juice in spontaneous non-insulin-dependent diabetes mellitus (NIDDM) rats. Journal of Health Science 2004; 50(6):674–678.
28. Cristani M, Arrigo MD, Mandalari G, Castelli F, Sarpietro MG, Micieli D et al.: Interaction of four monoterpenes contained in essential oils with model membranes: implications for their antibacterial activity. J Agric Food Chem 2007; 55: 6300-6308.
29. Kumar R, Shukla SK, Pandey A, Pandey H, Pathak A and Dikshit A: Dermatophytosis: Infection and Prevention -A Review. Int J Pharm Sci Res 2016; 7(8): 3218-25.doi: 10.13040/IJPSR.0975-8232.7(8).3218-25.
30. Bhatt D, Sachan AK, Jain S, Barik R: Studies on inhibitory effect of Eucalyptus oil on sebaceous glands for themanag ement of acne. Indian Journal of Natural Products and Res ources 2011;2 (3):345–349.
31. Sonboli A, Babakhani B, Mehrabian AR: Antimicrobial activity of six constituents of essential oil from Salvia. Z Naturforch 2006; 61: 160-164.
32. Qian H, Nihorimbere V: Antioxidant power of phytochemicals from *Psidium guajava* L.leaf. J. Zhejiang Univ Sci 2004; 5: 676-683.
33. Suzuki I, Kishida M, Okada M, Izawa M, Kurita F, Nakazono H, Koshibu T, Aoki T: A clinical evaluation of the effectiveness of Psidium guajava in atopic dermatitis. The International Congress of Allergy and Clinical Immunology, Abstract 2000; 713.
34. Jimenez EA, Rincon M, Pulido R, Saura-Calixto F: Guava fruit (*Psidium guajava* L.) as a new source of antioxidant dietary fiber. J. Agric. Food Chem 2001; 49: 5489–5493.

How to cite this article:
Pandey M, Qidwai A, Kumar R, Pandey A, Shukla SK, Pathak A and Dikshit A: Pharmacological and antibacterial aspect of *Psidium guajava* L. against acne vulgaris. Int J Pharm Sci Res 2017; 8(1): 145-50.doi: 10.13040/IJPSR.0975-8232.8(1).145-50.