Screening of anti-pustule plant metabolites from *Prosopis juliflora* and their combined anti-pustule activity with synthetic pimple creams

Rajadurai Jesudoss R P*, Lakshmipraba S, Gnanasaraswathi M, Ganesh Kumar S, Praveen Kumar T G
Department of Biotechnology, PSR engineering College, Sivakasi, Virudhunagar District, Tamilnadu.
***Corresponding Author: E.mail: jesuramnad@gmail.com**

ABSTRACT

Pimples emerged as a depressive threat among youth life style. Anti-pimple research, on pimple prevention & eradiation was always being hot core researches in cosmetic sector. Pimples are due to *Staphylococcus* sp infection with its increased microbial flora within the subcutaneous layer. Increased *Staphylococcus* sp biomass swells to develop as a pimple. Synthetic creams were available in large numbers for pimple reduction than herbal substitutes. Even though synthetic creams were effective in action they cause undesirable side effects like blackening & itching of skins, damage of tissues etc. In this study, plant metabolites from *Prosopis juliflora* possessing anti-pustule activity were screened and studied. Acetone extract of *Prosopis juliflora* (AEP) evidences effective anti-pustule activity by inhibiting *Staphylococcus* sp through well diffusion method. Minimum inhibitory concentration of AEP was found to be 0.75mg/ml. This inhibitory action was also confirmed by growth curve analysis. AEP was proved to have one or two compound through Thin Layer Chromatography. Functional groups in AEP were examined through FTIR. Combined anti-pustule activity of AEP with commercially available synthetic pimple creams was also studied, which ends in a conclusion that the activity of commercial synthetic creams has been increased by the addition of AEP.

KEYWORDS: *Prosopis juliflora*, Anti-pustule activity, *Staphylococcus* sp, Commercial pimple creams, FTIR

1. INTRODUCTION

Pimples were emerging threats in the young generation lifestyle. Pimples are due to *staphylococcus* infection, which causes puss formation underneath the subcutaneous layer (Dhillon and Krati, 2013). Puss accumulates as a swell like projection called pimples. Formulation of anti-pustule activity is being a core research in cosmetic sectors. Number of chemical compounds has been identified as potent pimple reduction compounds. Clindamycin phosphateis is one of the effective chemical compound used in cosmetic creams which resist the *Staphylococcus* sp, thus preventing pimples. Chemical bioactive compounds deserve its own demerits by inducing side effects such as rashes, redness on tissues and itches. Hence to replace these chemical compounds, bioactive herbal compounds possessing anti-pustule activity have been screened from various plant resources.

Prosopis juliflora, belongs to the family Leguminosae, is found in arid and semi-arid regions of Tamilnadu. The shrubs of *Prosopis juliflora* are highly esteemed for windbreaks, soil binders, sand stabilizers, living fences, fuel wood, bee plants and animal feed. These uses, together with fast growth (Wunder, 1966), drought resistance (Evans, 1982) and salt tolerance (Goyal et al., 1988) have lead to its introduction in many arid zones (Tene, 2007). A number of compounds have also been reported from this plant, the most common of these being steroids, tannins, leucoantho cyanidin and ellagic acid glycosides. Extracts of *Prosopis juliflora* seeds and leaves have several *in vitro* pharmacological effects such as antibacterial, antifungal, anti-inflammatory properties (Al and Al, 1999) (Kanthasamy, 1989). Flavonoids are water soluble phytochemical showing the antioxidant, anticancer and anti-inflammatory activities. These prevent cells from oxidative damage and carcinogenesis. Flavonoids are also used to cure some heart related diseases (Hussain et al., 2011). Flavonoids occur virtually in all parts of the plant, the root, heartwood, sapwood, bark, leaf, fruit and flower (Harborne, 1975). Alkaloids and their derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bactericidal activities (Harisaranraj et al., 2009). Alkaloid rich fractions of *Prosopis juliflora* are active antifungal and antibacterial agents (Aqeel et al., 1988) (Ahmad et al., 1989).

In this study *Prosopis juliflora* was taken as a plant source to screen its anti-pustule active compounds. Screened compounds were tested for their anti-pustule behavior by studying the inhibition levels of *Staphylococcus* sp. Combined effect of bioactive compound from *Prosobis juliflora* along with commercially available anti-pimple creams were studied. Clinigex & Clingel were chosen as syntheticanti-pimple creams for study.

2. MATERIALS AND METHODS

2.1. Sample Collection and Pretreatment: *Prosopis juliflora* leaves were collected from Rajapalayam, Virudhunagar (Dt), Tamil Nadu. Collected fresh leaves were allowed to dry in the sunlight for 3 days. Dried leaf sample was crushed into powder, with the help of mechanical crusher. Powdered sample was again dried in hot air oven at 40ºC for 5hrs to remove entire moisture.

2.2. Extraction of Anti-pustule plant metabolites: 5g of Powdered leaf was soaked in 25ml of solvent (Propanol, Cyclohexane, Chloroform, Ethanol, Acetone, Acetonitrile, Pyrimidine, Aniline) and smashed well to obtain the extract. The obtained extract was kept in boiling at a temperature below its respective solvents boiling point for about 5hr. The extract was filtered and air dried separately.

2.3. Isolation and Characterization of *Staphylococcus* sp: *Staphylococcus* sp were isolated from pimples (Puss). Loop of pimple puss was inoculated on nutrient agar (Himedia, India) and incubated at 37ºC for 24hrs. Single colony was isolated and maintained in a nutrient broth. Isolate was studied and characterized with the basic biochemical test.

2.4. Screening of Anti-pustule active plant metabolites

2.4.1. Well Diffusion Method: (Elsa and Valentin, 2013): Anti-pustule activity of *Prosopis juliflora* leaf extract was screened through well diffusion method. 18hrs old culture of *Staphylococcus* sp was coated on a nutrient agar (Himedia, India) plate with wells. 50µl of Plant leaf extract of varied solvents were pipetted into the well as test samples. Respective solvents were loaded separately as a positive control. Inoculated plates were incubated at 37ºC for 24 hrs in static condition. Standard antibiotic discs were tested for its inhibition efficiency along with the test sample of plant extract. After incubation, plates were analyzed for zone of inhibition by measuring the radius of zones appeared.

2.4.2. Growth Curve Analysis: Plant leaf extract of particular solvent exhibiting anti-pustule activity in well diffusion method were dried into powder and tested for its inhibitory efficiency against *Staphylococcus* sp through growth curve analysis. *Staphylococcus* sp was inoculated in nutrient broth (Himedia, India) of 250ml with 1% inoculum along with 1% of plant leaf extract. Inoculated nutrient broth without plant extract was chosen as a positive control.

2.4.3. Estimation of Minimum Inhibitory Concentration (MIC): Powdered extract exhibiting anti-pustule activity, was taken with different dilutions with di-methyl sulfoxide (Himedia, India) as 0.75mg/ml, 0.5mg/ml, 0.25mg/ml. Diluted extracts were loaded in nutrient agar (Himedia, India) by spread plate technique inoculated with *Staphylococcus* sp by well diffusion method. Minimum concentration at which zone of inhibition occurs was measured as MIC.

2.4.4. Thin Layer Chromatography (Nithya and Muthumary, 2011): TLC analysis were carried out on Merck 0.25 mm silica gel plates, developed in the following solvent system as different concentrations of acetone (100%, 75%, 50% & 25%) and mixed solvent of cyclohexane:acetone:methanol (3:3:3) and their retention factor (R_f) values were calculated. Then the TLC plates were exposed to UV rays to visualize UV activated flavanoids. Number of compounds in the acetone plant extract was estimated through thin layer chromatography.

$$Rf = \text{Distance travelled by the solute (cm)} / \text{Distance travelled by the solvent (cm)}$$

2.4.5. Fourier transform infrared analysis (Nithya and Muthumary, 2011): The IR spectra of the extracted compounds were measured on Shimadzu FT-IR 8000 series instrument. Extractedsample was grounded with IR grade potassium bromide (KBr) (1:10) pressed in to discs under vacuum using spectra lab pelletiser. The IR spectrum was recorded in the region 4000–400 cm^{-1} and the typical stretching frequency of the bioactive substance was recorded for further characterization analysis.

2.4.6. Combined anti-pustule activity of leaf extract with synthetic pimple creams: Anti-pustule activity of plant metabolite from the plant leaf extract was combined with the commercial synthetic anti-pimple creams containing clindamycin phosphate as their active ingredients i) Synthetic anti-pimple cream 1 (Clingel), ii) Synthetic anti-pimple cream 2 (Clinigex), and their combined effect was analyzed by well diffusion method and growth curve analysis. A mixture of 50mg AEP and 50mg of commercial pimple cream were taken as test sample. 50mg of commercial cream was kept as a positive control.

3. RESULTS

3.1. Characterization of isolate: On the basis of staining, the isolate was found to be gram positive bacteria with a cocci shaped structure. Its behavior towards biochemical test was listed in Table 1.

3.2. Screening of anti-pustule active plant metabolites: On initial screening of effective solvents for extracting potent anti-pustule compound from *Prosopis juliflora*, mid-polar solvent acetone shows effective inhibition which was evidenced in Table 2. Among various mid-polar solvent extracts, acetone extract of *Prosopis juliflora* (AEP) shows effective inhibition on *Staphylococcus sp* than the extracts of aniline, pyrimidine, acetonitrile (Table 2). This evidences AEP exhibits effective anti-pustule activity as shown in Fig 1. Minimum inhibitory concentration of AEP against *Staphylococcus* sp was found to be 0.75mg/ml were shown in Table 3. Growth curve analysis of AEP evidences 3 folds reduction of microbial growth, evidencing anti-pustule activity.

3.3. Combined anti-pustule action of AEP with synthetic anti-pimple creams: AEP on examined in combination with synthetic anti-pimple creams, shows effective inhibition than commercial synthetic anti-pimple creams as shown in Fig 2. AEP evidences 35% increased inhibition than commercial synthetic anti-pimple creams as evidenced from Table 4 and Fig 3.

3.4. Thin layer chromatography: Solvent system of varied concentration of acetone and mixed solvent system exhibits a single visual band with R_f around 0.6 on white light visualization as shown in Fig 4. UV visualization of TLC plate reveals the presence of two compounds with a close R_f value of 0.55 and 0.58 as on Fig 4 and Table 5.

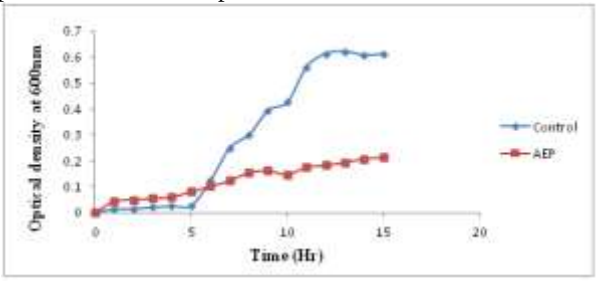


Figure.1.Anti-pustule activity by Growth curve analysis

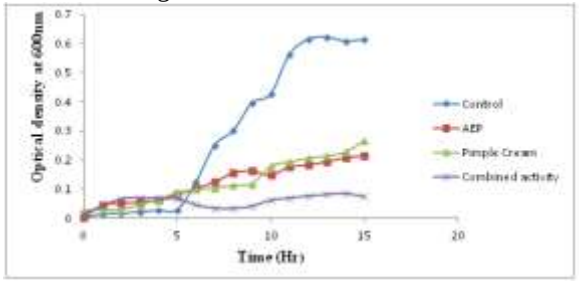


Figure.2.Combined Anti-pustule activity of AEP with synthetic pimple creams

Figure.2.Combined Anti-pustule activity of AEP with simple pimple creams

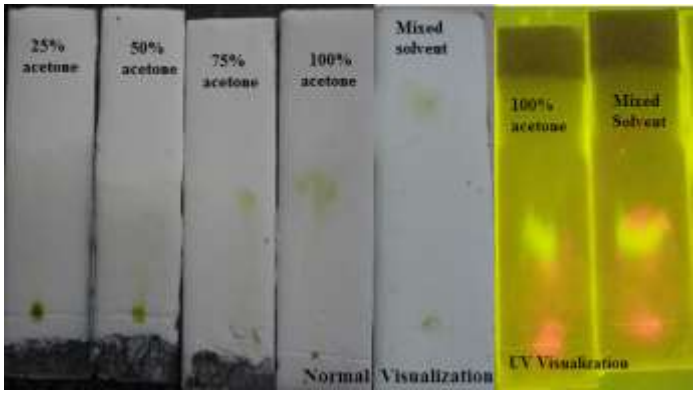
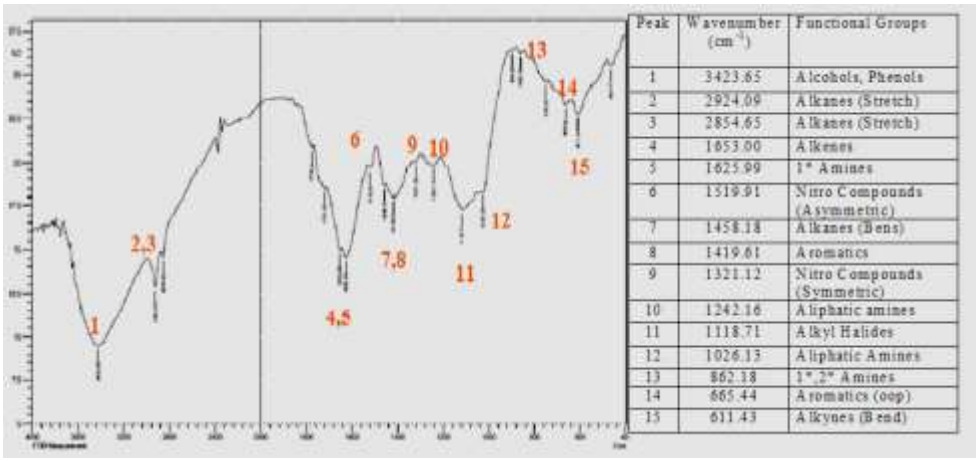


Figure.4.Thin Layer Chromatography



Peak	Wavenumber (cm^{-1})	Functional Groups
1	3423.65	Alcohols, Phenols
2	2924.09	Alkanes (Stretch)
3	2854.65	Alkanes (Stretch)
4	1653.00	Alkenes
5	1625.99	1° Amines
6	1519.91	Nitro Compounds (Asymmetric)
7	1458.18	Alkanes (Bens)
8	1419.61	Aromatics
9	1321.12	Nitro Compounds (Symmetric)
10	1242.16	Aliphatic amines
11	1118.71	Alkyl Halides
12	1026.13	Aliphatic Amines
13	862.18	1°,2° Amines
14	665.44	Aromatics (oop)
15	611.43	Alkynes (Bend)

Figure.5.FTIR Analysis

Table.1.Zone of inhibition for different polarity based solvents against *Staphylococcus* sp

Solvents	Type of solvents	Zone of inhibition in radius (mm)
Ethanol extract	Polar	6.5 ± 0.3
Ethanol		-
Acetone extract	Mid-Polar	18 ± 0.2
Acetone		-
Chloroform Extract	Non-Polar	-
Chloroform		-

Table.2.Anti-pustule activity of Mid-Polar solvents

Solvents	Types of solvents	Zone of inhibition in radius (mm)
Pyrimidine extract	Mid-Polar	12.5 ± 0.33
Pyrimidine		-
Acetone extract		20 ± 0.3
Acetone		-
Aniline extract		12.5 ± 0.3
Aniline		-
Acetonitrile extract		10 ± 0.25
Acetonitrile		-

Table.3. Minimum inhibitory concentration of AEP

AEP concentration (mg/ml of DMSO)	Zone of inhibition in radius (mm)
0.25	4 ± 0.5
0.5	4.5 ± 0.3
0.75	9 ± 0.6

Table.4.Combined Anti-pustule activity AEP (Acetone plant extract) with Synthetic pimple creams

Compounds	Zone of inhibition in radius (mm)
Acetone Extract of *Prosobis juliflora* (AEP)	20 ± 0.4
AEP + Synthetic pimple cream 1	26 ± 0.3
Synthetic pimple cream 1	18 ± 0.35
AEP + Synthetic pimple cream 2	28 ± 0.3
Synthetic pimple cream 2	16 ± 0.4

Table 5: Thin layer chromatography of AEP

Solvent system	No. of bands	R_f
100% Acetone	1	0.625
75% Acetone	1	0.6
50% Acetone	1	0.6
25% Acetone	0	-
Cyclohexane : Acetone : Methanol (3:3:3)	1	0.635

3.5. FTIR: FTIR analysis reveals about 15 peaks representing the available functional groups within the Acetone extract of *Prosopis juliflora*. Major peaks found in the region are3423.65, 2924.09, 1653.00, 1625.99, 1458.18, 1419.69, 1118.71 and 665.44 of wave number (Fig 5). The major functional groups are alcohols, phenols, aromatics, alkyl halides, alkanes and primary amines.

4. DISCUSSION

4.1. Anti-pustule activity of AEP: Effective inhibition of acetone, aniline, acetonitrile, and pyrimidine extracts (Table 1 & 2) evidences that solvents of partial polarity were effective in extracting potential anti-pustule compound from *Prosopis juliflora*. It also evidences ethanol extract (polar solvent) of *Prosopis juliflora* shows an effective inhibition against *Staphylococcus* sp (Sathiya M and Muthuchelian K, 2008). Over varied mid-polar solvents, acetone was highly potent in extracting a single compound of effective anti-pustule activity. AEP evidences 20mm of zone of inhibition for 0.75mg/ml concentration, but Sathiya M and Muthuchelian K, 2008 concluded that 50mg/ml of ethanolic extract of *Prosopis juliflora* shows only 10mm zone of inhibition. This evidences acetone extract of *Prosopis juliflora* act 2 folds effective and greater than ethanolic extract of the same. David O *et al.*, 2009 also reported that acetone extract of *Prosopis* family shows below 8 mm of zone of clearance, which was about 2 times lesser than this study result for acetone extract. Shachi singh *et al.*, 2011 reported that ethanolic extract of *Prosopis juliflora* evidences zone of 13mm against *Staphylococcus* sp. Thin layer chromatography evidenced the presence of single compound in acetone extract. Mixed solvent system also evidences two compounds in acetone extract.

4.2. Combined anti-pustule activity of AEP with synthetic anti-pimple cream: Commercially available pimple creams are effective inhibitors of *Staphylococcus* sp. Commercial pimple creams on treating against *Staphylococcus* sp shows an average 17mm zone of clearance. Addition of AEP along commercial synthetic creams has evidenced an increased average zone of inhibition as 27mm. These results conclude that AEP has potent compound against *Staphylococcus* sp, which can be effectively used with commercial pimple creams to enhance their anti-pustule activity.

4.3. Growth curve analysis of *Staphylococcus* sp aided AEP & Pimple creams:

4.3.1. AEP activity: AEP controls the growth of *Staphylococcus* 3 fold times than the control. AEP dominantly resist log phase of the system, thus limiting the biomass population. Once multiplication of pathogen has been resisted, chance of persistent infection will be eradicated. This indicates that AEP acts at the initial stage of pimple formation and prevents it by resisting early and late log phase of the pathogen.

4.4. Combined activity: Commercial synthetic anti-pimple creams resist early log phase potentially and exhibit partial resistance at late log phase of the pathogen life. Both AEP and synthetic anti-pimple creams are similar in action in inhibiting the pathogen. Synthetic anti-pimple creams also evidences 2 folds complete reduction of microbial biomass as compared with control. Combined action of AEP and synthetic anti-pimple creams acts 4 folds reduction of microbial biomass. AEP combined with synthetic anti-pimple creams induces early lag phase but totally resist and control microbial load in log and subsequent stationary phase.

Mechanism of AEP & synthetic anti-pimple creams in anti-pustule action was evidenced as complete fall of log phase of the microbial system.

4.5. TLC and FTIR analysis: On TLC analysis the number of compounds in the acetone extract was analysed. White light visualization evidences 1 band of R_f value around 0.6 in all eluting solvent system (i.e) different concentration of acetone and mixed solvent system. UV visualization evidences two bands in green and pink color of R_f valueas 0.58 and 0.56 respectively. Band of R_f value 0.58 of UV visualization and band of R_f0.6 of white light visualization are seem to be same band containing same compound. So it is evidenced that the acetone extract of *Prosopis juliflora* found to contain two compounds from the obtained TLC results.

FTIR analysis concludes with a graph of 15 major peaks indicating the vital functional groups. Secojuliprosopinal (Choudhary MI et al, 2005), Juliflorine, Juliprosine (Ahmad VU et al., 1989), Isojuliprosine, Juliprosinene (Hiroshi N et al., 2004) are the major alkaloids found in leaf of *Prosopis juliflora* (Muhammad Ibrahim et al., 2013). Structures of the alkaloids include functional groups such as primary amines, alcohols, alkenes, aromatics. FTIR analysis of AEP reveals the presence of above mentioned functional groups in the wavenumber range 3423.65, 2924.09, 1625.99, 1419.61, 1118.71 of phenols, alkanes, primary amines, aromatics, alky halides indicating that AEP might have contain Juliflorine, Juliprosine, Juliprosinene. Thus this acetone extract was rich in alkaloids (DhananjayaSeturamanPrabha et al., 2014).

5. CONCLUSION

Acetone extract of *Prosopis juliflora* found to contain potent anti-pustule active plant metabolite. Results of combined anti-pustule activity of AEP along with Synthetic anti-pimple creams reveals a fact that AEP can be strongly considered as an effective anti-pustule compound while formulating anti-pimple creams for immediate and effective resistance of *Staphylococcus* sp in preventing and controlling pimples.

ACKNOWLEDGEMENT

I sincerely thank the management, HOD & faculty members of Department of Biotechnology, PSR Engineering College for their valuable guidance throughout this process. I also thank all my fellow friends who have been helped extensively in this study.

REFERENCE

Ahmad VU, Azra S and Qazi S, Alkaloids from the leaves of *Prosopis juliflora*, Journal of Natural Products, 52(3), 1989, 497-501.

Al ShakhHamed WMA and Al Jammas MA, The antimicrobial activity of alkaloidal fraction of *Prosopis juliflora*, Iraqi Journal of Veterinary Sciences, 12(2), 1999, 281-287.

Allen ON and Allen EK, The Leguminosae: A Source Book for Characteristics, Uses, and Nodulation. The University of Wisconsin Press, Madison, USA, 1981.

Aqeel A, Ahmad KA, Khan S, Qazi VU, Antibacterial activity of an alkaloidal fraction of *Prosopisjulflora*. Fitoterapia, 59(6), 1988, 481-484.

Choudhary MI, Nawaz SA, Zaheer-ul-Haq, Kamran MA, Nabeel MG, Arif ML, Jalil S, Khalid A, Ahmed A, Rode BM, Rahman A, Gilani A and Ahmad VU, Juliflorine: A potent natural peripheral anionic-site-binding inhibitor of acetylcholinesterase with calcium-channel blocking potential, a leading candidate for Alzheimers disease therapy. Biochemical and Biophysical Research Communications, 332, 2005, 1171-1179.

David O, Gema L, Guadalupe Valladares Cisneros, Ismael León Rivera, Oscar Dorado, Ramírez, Ma., Salinas Sánchez.Víctor M. Navarro García1, Antimicrobial activity of medicinal plants from the huautla sierra biosphere reserve in Morelos (México). Polibotanica, Núm. 28, 2009, 213-225.

DhananjayaSeturamanPrabha, Hans-UweDahms, PrabhaMalliga, Pharmacological potentials of phenolic compounds from Prosopis spp.-a review, Journal of Coastal Life Medicine, 2(11), 2014.

Dhillon KS and Krati R Varshney, Study of Microbiological Spectrum in Acne Vulgaris: An *In Vitro* Study. Scholars Journal of Applied Medical Sciences, 1(6), 2013, 724-727.

Elsa Lycias Joel, ValentinBhimba B, A secondary metabolite with antibacterial activity produced by mangrove foliar fungus *Schizophyllum commune*, International Journal of Chemical, Environmental & Biological Sciences, 1(1), 2013.

Evans J, Planetary Forestary in the Tropics. Oxford: Clarendon Press, 394, 1982.

Goyal SP, Bohra HC, Ghosh PK, Parakash I. Journal of Arid Environments, 14, 1982, 285.

Harborne JB, The Flavonoids, Academic Press, New York, Part-1, Chapters 1-11, 1975.

Harisaranraj R, Suresh K and Saravanababu S, Evaluation of the Chemical Composition *Rauwolfia serpentine* and *Ephedra vulgaris*. Advances in Biological Research, 3(5-6), 2009, 174-178.

Hiroshi N, Eri N, Syuntaro H, Yoshiharu F, Kosumi Y, Hideyuki S, Koji H, Growth inhibitory alkaloids from mesquite (*Prosopis juliflora*, Swartz DC.) leaves, Phytochemistry, 65(5), 2004, 587-91.

Hussain J, Khan FU, Riazullah Z Muhammad, Rehman NU, Shinwari ZK, Khan IU, Zohaib M, Imad-ud-Din and Hussain SM, Nutrient Evaluation and Elemental Analysis of four selected medicinal plants of Khyber PakhtoonKhwa, Pakistan. Pakistan Journal of Botany, 43(1), 2011, 427-434.

Kanthasamy A, Subramanian S, Govindasam S, Bactericidal and fungicidal effects of Prosopisjulifloraalkaloidal fraction. Indian Drugs, 26, 1989, 390-394.

Muhammad ibrahim, Muhammad Nadir, Amanatali, Viqaruddinahmad and Munawwerrasheed, Phytochemical analyses of *prosopisjulifloras*wartz dc. Pakistan Journal of Botany, 45(6), 2013, 2101-2104.

Nithya K and Muthumary J, Bioactive metabolite produced by *Phomopsis sp*., Anendophytic fungus in *Allamanda cathartica linn*. Recent Research in Science and Technology, 3(3), 2011, 44-48.
Sathiya M and Muthuchelian K, Investigation of Phytochemical Profile and Antibacterial Potential of Ethanolic Leaf Extract of *Prosopis juliflora* DC. Ethnobotanical Leaflets, 12(15), 2008, 1240-45.
Shachi Singh et al., Antibacterial properties of Alkaloid rich fractions obtained from various parts of *Prosopis juliflora*. International Journal of Pharma Sciences and Research, 2(3), 2011, 114-120.
Tene V, Malag´on O, Finzi PV, Vidari G, Armijos C, Zaragoza T, An ethnobotanicalsurvey of medicinal plants used in Loja and Zamora-Chinchipe, Ecuador. Journal of Ethnopharmacology, 111, 2007, 63-81.
Warag MAO, Journal of Arid Environments, 27, 1994, 79-84.
Wunder WG, *Prosopis Juliflora* (*P. africana*): In the Arid Zone of Sudan, Pamphlet of the Forest Research Education Project, 1966.