IJPSR (2018), Volume 9, Issue 6 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 24 August, 2017; received in revised form, 23 December, 2017; accepted, 30 December, 2017; published 01 June, 2018

ANTIMICROBIAL ACTIVITY OF *EXACUM LAWII* EXTRACTS AND VOLATILE OIL AGAINST OCULAR INFECTION

Sonam Sharma [1], Gopal Nath [2] and Siva Hemalatha[*1]

Department of Pharmaceutical Engineering and Technology [1], Indian Institute of Technology, Department of Microbiology [2], Institute of Medical Sciences, Banaras Hindu University, Varanasi - 221005, Uttar Pradesh, India.

Keywords:

Exacum lawii, Phytochemical screening, MBC, MFC, Ocular infection

Correspondence to Author:
Dr. Siva Hemalatha

Department of Pharmaceutical Engineering and Technology, Indian Institute of Technology, Banaras Hindu University, Varanasi - 221005, Uttar Pradesh, India.

E-mail: shemalatha.phe@itbhu.ac.in

ABSTRACT: *Exacum lawii* is folklore medicinal herb, boiled with oil and used traditionally for ocular infection. The aim of the present study is to evaluate the *in-vitro* antibacterial and antifungal activity against ocular infection. Ethanolic extract, petroleum ether extract and volatile oil were extracted from *Exacum lawii* whole plant. Antibacterial activity and antifungal activity against strains causing ocular infection were evaluated and Himalaya Optha-care Eye Drop was used as standard. The antimicrobial susceptibility was performed by agar disc diffusion assay method. The minimum bactericidal concentration (MBC) and minimum fungicidal concentration were determined for each extract and volatile oil by subculturing the samples. The volatile oil and the extracts of *Exacum lawii* showed bactericidal and fungicidal activities against some strains of bacteria and fungi causing ocular infection. Minimum bactericidal concentration for volatile oil was found in range of 3.125 mg/ml to 12.25 mg/ml, for ethanolic extract 6.25 mg/ml to 25 mg/ml and for petroleum ether extract 25 mg/ml to 50 mg/ml. Minimum fungicidal concentration of volatile oil was found to be 3.125 mg/ml to 12.5 mg/ml, for ethanolic extract 12.5 mg/ml to 25 mg/ml and for petroleum ether extract 25 mg/ml to 50 mg/ml. This study suggested that the volatile oil is possess potent antimicrobial activity by inhibiting the growth of bacteria and fungi causing ocular infection significantly than the ethanolic and petroleum ether extract of *Exacum lawii*.

INTRODUCTION: There are many advances in antibiotic therapy still infectious complications remain an important cause of mortality and morbidity among hospitalized patients. The use of these synthetic antibiotics may subject the patient to a greater risk, due to the possibility of the drugs to associate with more harmful side effects.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.9(6).2266-72

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

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(6).2266-72

Advances in identifying novel moiety from natural products with antimicrobial activities and it will lead to expand chemical diversity of antimicrobial agents following with chemical leads for new drugs [1]. There are majority of modern medications which were derived initially from ancient herbal medicines.

From the centuries, medicinal plants have been used as remedies for human diseases as they contain components of therapeutic value. There are numerous natural products from plant sources reported to have anti-bacterial, anti-fungal and anti-protozoal activities that can be used either locally or systemically [2]. Several medicinal plants having volatile oils, polyphenols and alkaloids as major

constituents serve as phytomedicines are being utilized for their antimicrobial activity. These are significantly effective in the treatment of infectious diseases also simultaneously mitigating major side effects that are often associated with synthetic anti-microbials [3]. *Exacum lawii* C.B. Clarke in Hook species of genus Exacum is small annual herb commonly distributed in the Western peninsula, Western coast region of India and Southern part India mainly Mysore and Coimbatore. It is endemic to Jarandeshwar hill from Satara district, Maharashtra and Western ghat of Karnataka.

The common English name of *Exacum lawii* is Law's Persian violet. It is locally known as Lahanchirayata in Maharashtra, Manali in Malayalam, Marukozhunthu in Tamil. This bitter herb is an annual, glabrous, small erect herb rarely reaching upto 15 cm tall. Flowers are bluish-purple. The whole plant has been used as folk medicinal remedy for treatment of kidney disorders and eye diseases [4 - 13].

Indian population is vulnerable to eye infections by virtue of subtropical climate of India. Ocular infections are among the most common disease processes that can affect the normal eye. They can be mild or severe ocular infections, mild infection are essentially self-limiting, and severe infections are sight-threatening. Severe condition requires aggressive treatment to preserve sight [14 - 15]. Infection of the conjunctiva leads to inflammation is known as conjunctivitis and is characterized by dilatation of the conjunctival vessels, resulting in edema and hyperemia of the conjunctiva, typically associated with discharge from eyes [16].

The prevalence of conjunctivitis varies according to the underlying cause, which may be influenced by the patient's age, as well as the season of the year. Bacterial conjunctivitis is the second most common cause after viral conjunctivitis and is responsible for the majority (50%-75%) of cases in children, viral infection mainly affects the adult population. Allergic conjunctivitis is the most frequent cause, affecting 15 % to 40 % of the population, and is observed more frequently in spring and summer. Bacterial conjunctivitis can be divided into three groups: hyperacute, acute, chronic and severe [17 - 22]. Acute bacterial conjunctivitis may be acute papillary, hyperacute purulent, or acute membranous conjunctivitis. Purulent conjunctivitis is caused mainly due to *Haemophilus influenza, Neisseria* or *Staphylococcus* infection. Other form of intraocular infection of the vitreous compartment of the eye along with retinal and uveal coats caused endophthalmitis. Organisms causing acute endo-phthalmitis include mainly *Staphylococcus aureus*, *Pseudomonas pneumonia*, *Pseudomonas aeruginosa*. Postoperative endophthalmitis can occur after any intraocular surgery [23]. Pseudomonas species is reported to be the most commonly isolated organism in keratitis associated with daily or extended wear soft contact lenses [24].

MATERIAL AND METHODS:

Plant Material: The fresh plant samples were collected in year of 2016 in the month of August-October from Mahabaleshwar, Maharashtra, India. The plant was authenticated by Dr. N. M. Dongarwar, Assistant Professor, Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, India and quality control standardisation was previously done. Voucher specimen (Cog/EL/2014-15) was deposited for future reference in Pharmacognosy laboratory of Department of Pharmaceutical Engineering and Technology, Indian Institute of Technology (Banaras Hindu University), Varanasi, India [25].

Plant Extracts Preparation and Volatile Oil Isolation: The plants were freshly collected, thoroughly washed and shade dried at room temperature to prevent deterioration of thermo labile phytoconstituents. The dried plant was coarsely powdered using mechanical grinder, passed through 60 mesh sieve size and stored at room temperature until extraction. The ethanolic extract (ELE) and petroleum ether extract (PLE) were prepared by cold maceration method. Both the extracts were concentrated under vacuum rotary evaporator (IKA Germany) and stored in desiccator until use. The volatile oil was isolated using Clevenger apparatus (hydro distillation method). Powdered drug (20 gram) was taken in distillation flask. The oil collected in graduated tube was 2 ml. The oil was stored in closed vial at room temperature.

Phytochemical Screening and GCMS Analysis: Preliminary phytochemical screening of ELE and PLE were done earlier by standard protocol along with quantitative estimation of phenolic content,

flavonol content and alkaloidal content. Fatty acid profile and Vitamin composition was also determined using Association of Official Analytical Chemists Methods. HPLC standardization with swertiamerin and ursolic acid along with GC-MS analysis was performed to identify some constituents of the ethanolic extract of *Exacum lawii*

Test Microorganisms: Microorganisms were used to determine the antimicrobial potential of the volatile oil and the plant extracts of *Exacum lawii*. Pathogenic bacterial strains causing ocular infection were: *Echerichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Pseudomonas pneumonia*, *Haemophilus influenza*, *Neisseria gonorrhoeae*, and *Chlamydia trachomatis*. Pathogenic fungal strains causing ocular infection were *Candida albicans*, *Candida tropicalis*, *Aspergillus*, *Fusarium*. All cultures were obtained from American Type Culture Collection (ATCC) and preserved at the Department of Microbiology, Institute of Medical Sciences, BHU, Varanasi, India. The fresh bacterial broth cultures were prepared before the screening procedure.

Antimicrobial Susceptibility Test: The disk diffusion method for antimicrobial susceptibility test was initially performed to determine the antibacterial activities of the *Exacum lawii* against pathogenic bacteria causing ocular infection. Antibacterial and antifungal activities were screened by disc diffusion method [26 - 28]. The stock solution was prepared by dissolving volatile oil and 1 gm of plant extract in 100 ml of nuclease free water. The stock solution was serially diluted to prepare various dilutions. The 5 µl from each dilution was dispensed on a sterile disc of Whatman's filter paper no.1 of 6 mm diameter for susceptibility testing. Muller Hinton agar (MHA) plates were prepared by pouring 15 ml of molten media into sterile petriplates.

The freshly grown colonies were inoculated in normal sterile saline solution to achieve desired concentration (10^7 cfu/ ml). The bacteria were streaked in a radial pattern on the agar plates (Remel™, Thermo Fisher Scientific, USA) (Meyer and Afolayan, 1995). The plates were allowed to dry. The prepared solutions of volatile oil and extract were put on 6 mm sterile disc of Whatman filter paper no. 1. The disc was then placed on the surface of the plate containing medium and the compound was allowed to diffuse for 5 min and the plates were kept for incubation at 37 °C for 24 hours for bacteria and 48 hour at 25 °C for fungal agents. At the end of incubation, zone of inhibition were examined. Experiment was done in triplicate.

Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC): Standard (Himalaya Optha-care Eye Drop) and test sample of plant extracts and volatile oil were prepared using serial dilution method according to the National Committee for Clinical Laboratory Standards, 2000 (NCCLS, 2000). The test was carried out in 96-well microtiter plates. Equal volumes of each dilution of standard, test sample and nutrient broth were mixed in wells of a microtiter plate. Specifically, 0.1 ml of standardized inoculum (2×10^7 cfu/ml) was added to each well of the microtiter plate. The plates were incubated aerobically at 37 ^oC for 18-24 hours for bacteria and 48 hours at 25^oC for fungal growth. The lowest concentration (highest dilution) of the standard and test sample that produced no visible bacterial growth (no turbidity) when compared with the control was regarded as the MIC.

Further, the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined by sub-culturing the test dilution from wells of microtitre plate on to a fresh drug-free solid medium and incubated further for culture. The highest dilution that yielded no bacterial or fungal colony was taken as the MBC and MFC, respectively [29 - 30].

Statistical Analysis: Analysis of variance (ANOVA) had done by one-way ANOVA followed by Newman-Keuls multiple comparison tests to determine the statistical significance between different groups. A difference in the mean values of $P < 0.05$ had been considered to be statistically significant. For all statistical analyses GraphPad Prism 5.0 software (GraphPad Software, Inc., La Jolla, CA, USA) had been used.

RESULTS:

Phytochemical Screening and GCMS Analysis: The preliminary phytochemical screening of ELE and PLE evaluated the presence of alkaloids, flavonoids, phenolic compounds, terpenoids, bitter

compounds, steroids and coumarins. The total phenolic content for ELE (12.21 ± 0.58), for PLE (1.45 ± 0.15), Flavonoid content for ELE (21.86 ± 0.22), for PLE (1.56 ± 0.10), flavonol content for ELE (15.53±0.05), for PLE (0.91 ± 0.02) and alkaloidal content for ELE (35.59 ± 0.13), for PLE (6.53 ± 0.15). Fatty acid profile showed different concentration of saturated fatty acids, poly unsaturated fatty acids, monounsaturated fatty acids, trans-fatty acids. Palmitic acid (1.37 gm/100 gm), stearic acid (0.41 gm/100 gm), oleic acid (0.41 gm/100 gm), linoleic acid (1.11 gm/100 gm) and alpha-Linolenic acid (0.76 gm/100 gm) along with other fatty acids. From the results Vitamin C was found to be in higher amount 6.11 mg/100 gm as ascorbic acid, Vitamin E was 5.06 mg/100 g and Vitamin A was 5.02 mg/100 gm. The concentration of swertia-marin and Ursolic acid in ethanolic extract of *Exacum lawii* calculated was 119.59 mg/gm and 5.34 mg/gm respectively. GC-MS analysis of ethanolic extract lead to the identification of palmitic acid (RT, 33.49), ethyl iso-allocholate (RT, 22.63), echitamine (RT, 28.43), 8.11-Eicosadienoic acid, methyl ester (RT, 331.01), ascorbic acid 2,6-dihexadecanoate (RT, 33.49), dodecanoic acid ethyl ester (rt, 34.07), docosanoic acid, 1,2,3-propanetriyl ester (RT, 45.679), trilinolein (RT, 36.61), 2H-Tetrazol-5-amine, 2-(phenyl methyl)- (RT, 27.15) and tri-linolein (RT, 36.61) (**Fig. 1-3**).

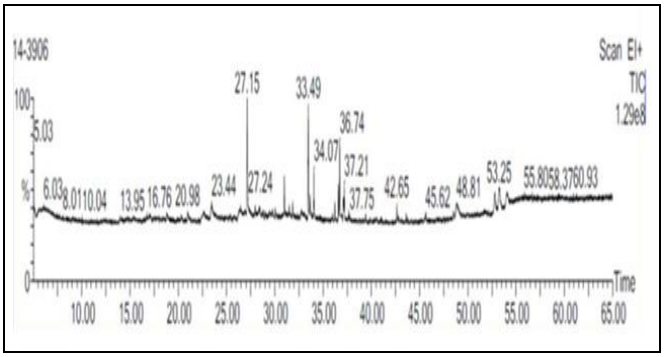

FIG. 1: GC-MS CHROMATOGRAM OF ETHANOLIC EXTRACT OF *EXACUM LAWII*

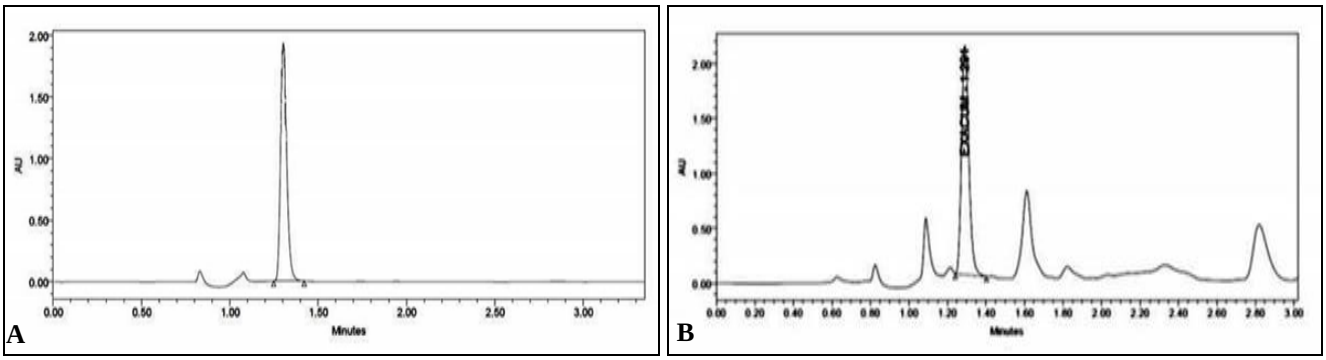

FIG. 2: A) HPLC CHROMATOGRAM SHOWING PEAK OF STANDARD SWERTIAMERIN, B) HPLC CHROMATOGRAM OF ETHANOLIC EXTRACT OF *EXACUM LAWII* SHOWING PEAK OF SWERTIAMERIN

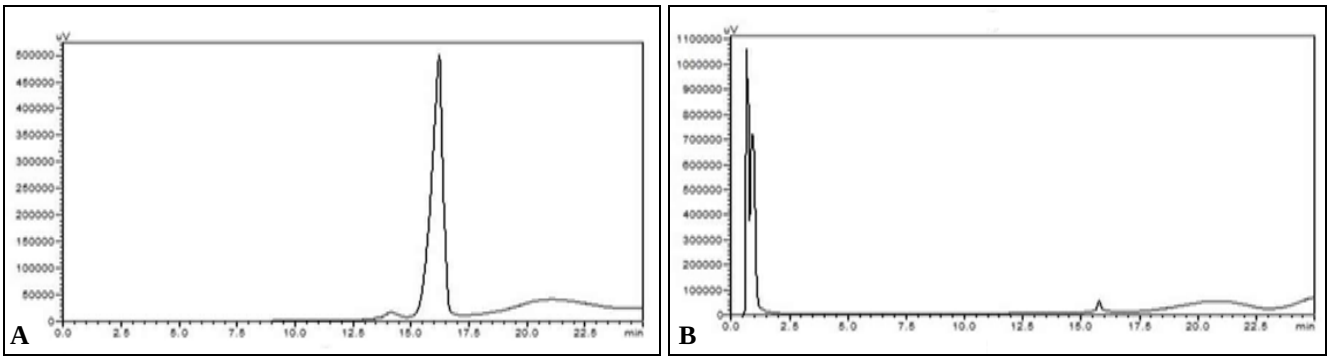

FIG. 3: A) HPLC CHROMATOGRAM SHOWING PEAK OF STANDARD URSOLIC ACID, B) HPLC CHROMATOGRAM OF ETHANOLIC EXTRACT OF *EXACUM LAWII* SHOWING PEAK OF URSOLIC ACID

Antimicrobial Susceptibility Test: Activities of different extracts against the test organisms were expressed as zone of inhibition (in mm). The zone of Inhibition for bacterial strain causing ocular infection for volatile oil ranged between 10.05 ± 0.78 to 21.15 ± 1.34, for ethanolic extract 5.20 ± 0.85 to 18.05 ± 0.64 and for petroleum ether extracts 5.10 ± 0.43. The zone of Inhibition for pathogenic fungal strain causing ocular infection ranges between 6.95 ± 0.63 to 18.20 ± 0.42 (volatile oil), 4.95 ± 0.63 to 11.65 ± 1.34 (ethanolic extract) and 0 to 8.00 ± 0.7 (petroleum ether extract) (**Fig. 4** and **5**).

Minimum bactericidal concentration (MBC) and Minimum fungicidal concentration (MFC): MBC and MFC for volatile oil of *Exacum lawii* against various bacterial and fungal strains causing ocular infection ranged between 3.125 mg/ml to 12.25 mg/ml and 3.125 mg/ml to 12.5 respectively.

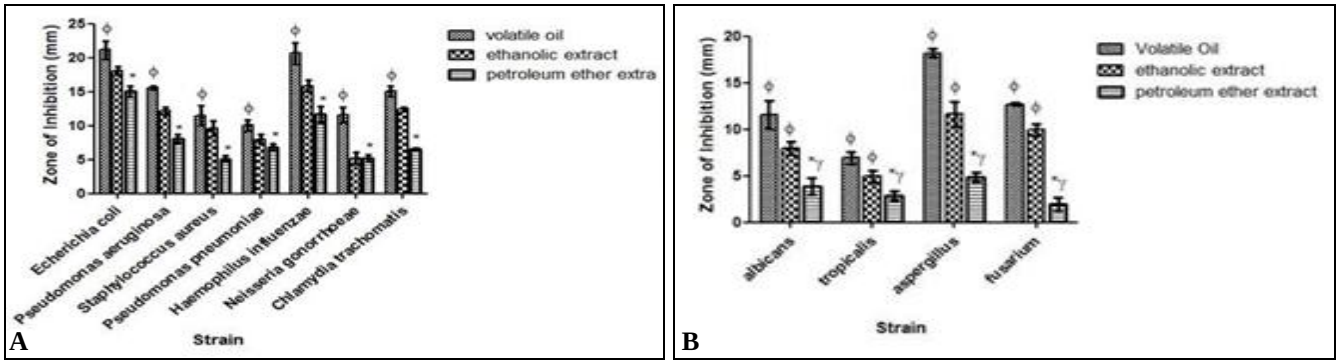

FIG. 4: ZONE OF INHIBITION FOR VOLATILE OIL AND EXTRACTS OF *EXACUMLAWII* AGAINST a) PATHOGENIC BACTERIAL STRAINS CAUSING OCULAR INFECTION, b) PATHOGENIC FUNGAL STRAINS CAUSING OCULAR INFECTION

Values are expressed as mean ± SD. *P<0.05, comparing Zone of Inhibition for volatile oil; Φ P<0.05, comparing Zone of Inhibition for petroleum ether extract of *Exacum lawii*;γ P<0.05, comparing Zone of Inhibition for ethanolic extract of *Exacum lawii*. One-way ANOVA followed by Newman–Keuls multiple comparison tests.

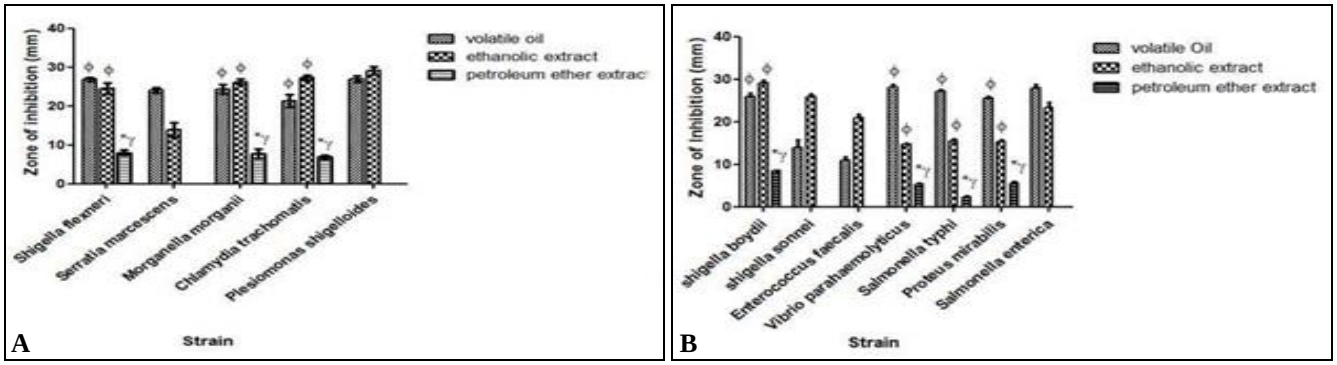

FIG. 5: ZONE OF INHIBITION FOR VOLATILE OIL AND EXTRACTS OF *EXACUMLAWII* AGAINST SOME PATHOGENIC BACTERIAL STRAINS

Values are expressed as mean ± SD. *P<0.05, comparing Zone of Inhibition for volatile oil; Φ P<0.05, comparing Zone of Inhibition for petroleum ether extract of *Exacum lawii*; γ P<0.05, comparing Zone of Inhibition for ethanolic extract of *Exacum lawii*. One-way ANOVA followed by Newman–Keuls multiple comparison tests.

TABLE 1: MIC AND MBC (mg/ml) VALUES OF *EXACUM LAWII* EXTRACTS AND VOLATILE OIL AGAINST BACTERIAL STRAIN CAUSING OCULAR INFECTION

Bacterial strain	Gram +ve/ -ve	MIC/MBC (mg/ml)			
		Volatile oil	Ethanolic extract	Petroleum ether extract	Standard
Echerichia coli	-ve	3.125/6.25	6.25/12.25	25/50	1.56/3.125
Pseudomonas aeruginosa	- ve	3.125/3.125	6.25/6.25	25/25	3.125/3.125
Staphylococcus aureus	+ ve	3.125/3.125	6.25/6.25	25/25	1.56/3.125
Pseudomonas pneumoniae	+ ve	6.25/12.25	12.25/25	25/25	3.125/6.25
Haemophilus influenzae	- ve	3.125/3.125	12.25/12.25	25/25	1.56/3.125
Neisseria gonorrhoeae	- ve	6.25/6.25	12.25/25	25/50	3.125/6.25
Chlamydia trachomatis	- ve	6.25/12.5	25/25	25/50	3.125/6.25

TABLE 2: MIC AND MFC (mg/ml) VALUES OF *EXACUM LAWII* EXTRACTS AND VOLATILE OIL AGAINST FUNGAL STRAIN CAUSING OCULAR INFECTION

Fungal strains	MIC/MFC (mg/ml)			
	Volatile oil	Petroleum ether extract	Ethanolic extract	Standard
Candida albicans	3.125/3.125	25/25	12.5/12.5	1.56/3.125
Candida tropicalis	12.5/12.5	25/25	12.5/12.5	6.25/12.5
Aspergillus keratitis	6.25/6.25	50/50	25/25	3.125/6.25
Fusariumdimerum	6.25/6.25	25/50	12.5/12.5	1.56/6.25

MBC and MFC ranged between 6.25 mg/ml to 25 mg/ml and 12.5 mg/ml to 25 mg/ml for ethanolic extract respectively and 25mg/ml to 50 mg/ml to 25 mg/ml to 50 mg/ml for petroleum ether extract respectively, against various bacterial and fungal strain causing ocular infection. MBC and MFC for standard (Himalaya Optha-care Eye Drop) against various bacterial and fungal strains causing ocular infection ranged from 1.56 mg/ml to 6.25 mg/ml (**Table 1** and **2**).

DISCUSSION AND CONCLUSION: Ethno-botanical study leads to approximately 74 % of the pharmaceutically active plant derived compounds. Potentially active compounds can be identified by investigating chemical composition and ingredients

of traditional medicinal plants. The aromatic oil extracted from the wide range of plants is reported to possess antimicrobial activity [31 - 32]. Since, the folklore medicinal plant, *Exacum lawii* has been traditionally used for eye problems and kidney problems. The plant has been standardised and reported to have swertiamerin with other bitter phytoconstituents.

In the present study, the antimicrobial activity of volatile oil and extracts of *Exacum lawii* against pathogenic strains of bacteria and fungi was determined by measuring the diameter of the inhibition zones around the discs. The MBCs and MFCs were also calculated by micro dilution method. Study showed that whole plant of *Exacum lawii* has a broad spectrum antimicrobial activity against pathogenic bacteria and fungi causing ocular infection. The MBCs and MFCs for volatile oil are found to be lower than ELE and PLE. The study justified the traditional use for eye problems by showing antimicrobial activity against pathogens causing ocular infection. The *Exacum lawii* extract is rich in phytoconstituents. The most of the secondary metabolites like phenols, alkaloids, flavonoids possess antimicrobial activity [34].

GCMS results confirmed that ELE contains polyunsaturated fatty acids, indole alkaloids also tetrazole derivative which are reported to have antimicrobial activity [35]. The bitter compounds and ursolic acid also play important role in antimicrobial activity [36 - 37]. The swertiamerin is also reported to have antibacterial activity [38]. They all may play very essential role in diverse therapeutic activity. The results obtained from the present study confirmed that *Exacum lawii* is rich in phytoconstituents that are able to combat the microbial defences. Henceforth, the results justify that further investigation should be performed to investigate the composition of volatile oil extracted from *Exacum lawii*. It will strengthen its potential of *Exacum lawii* as a novel antibacterial and antifungal agent.

ACKNOWLEDGEMENT: Department of Pharmaceutical Engineering and Technology, IIT (B.H.U) Varanasi is highly acknowledged for providing financial support to Ms. Sonam Sharma as Teaching Assistantship.

CONFLICT OF INTEREST: Nil

REFERENCES:

1. Nascimento GGF, Locatelli J, Freitas PC and Silva GL: Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Braz J Microbiol 2000; 31(4): 247-56.
2. Cowan MM: Plant products as antimicrobial agents. Clin Microbiol Rev 1999; 12(4): 564-82.
3. Kaushik P and Goyal P: *In-vitro* evaluation of *Datura innoxia* (thornapple) for potential antibacterial activity. Indian J Microbiol 2008; 48(3): 353-7.
4. Hooker JD: The Flora of British India. L. Reeve and Co. London, 41885.
5. Gamble JS: The Flora of Presidency of Madras. Adlard and Son Ltd. London, Vol. 2, 1923; 612-615.
6. Kirtikar KR and Basu BD: Indian Medicinal Plants. Bishen Singh Mahendra Pal Singh, Allahabad, Vol. 3(2), 1935; 1654-1655.
7. Chopra RN, Nayar SL, Chopra IC, Asolkar LV and Kakkar KK: Glossary of Indian medicinal plants. New Delhi: Council of Scientific and Industrial Research, 1956; 116.

8. Linnaeus C: Dissertatio Dassow. C.M. Nova Plantarum Genera, Stockholm, Sweden, 1747a.
9. Linnaeus C: Flora Zeylanica. Sumtuet Literis Laurentii Salvii, Holmiae, 1747b.
10. Struwe L, Hagen KB, Kadereit JW, Klackenberg J, Nilsson JS, Thiv M and Albert VA: Systematics, character evolution, and biogeography of Gentianaceae, including a new tribal and subtribal classification. In: Struwe L, Albert V.A. (eds). Gentianaceae-Systematics and natural history. Cambridge University Press, Cambridge, 2002.
11. Clarke CB: Gentianaceae in: J.D. Hooker (ed.), 1883.
12. Damaji B and Khandekar VP: Endemic plants of Maharashtra state at Jarandeshwar hill of Satara district (Maharashtra). Indian Streams Research Journal, 2011; 1: 1-3.
13. Vinayaka KS, Ashwini HS, Prashith KTR and Krishanamurthy YL: Traditional utilization and phyto-chemical analysis of medicinal herb Exacum Linn. From central Western Ghats. Asian Pacific Journal of Health Sciences, 2016; 3(1): 161-164.
14. Sharma S: Ocular infections: Research in India. Indian J Med Microbiol, 2010; 28: 91-4.
15. Traci LT, Sharon SC and Jack ET: Anterior ocular infections: An Overview of pathophysiology and treat-ment. Ann Pharmacother, 2000; 34: 235-46.
16. Leibowitz HM: The red eye. N Engl J Med, 2000; 343(5): 345-351.
17. Hørven I: Acute conjunctivitis: a comparison of fusidic acid viscous eye drops and chloramphenicol. Acta Ophthalmol (Copenh) 1993; 71(2): 165-168
18. Stenson S, Newman R and Fedukowicz H: Laboratory studies in acute conjunctivitis. Arch Ophthalmol 1982; 100(8): 1275-1277.
19. Rönnerstam R, Persson K, Hansson H and Renmarker K: Prevalence of chlamydial eye infection in patients attending an eye clinic, a VD clinic, and in healthy persons. Br J Ophthalmol 1985; 69(5): 385-388.
20. Harding SP, Mallinson H, Smith JL and Clearkin LG: Adult follicular conjunctivitis and neonatal ophthalmia in a Liverpool eye hospital. Eye (Lond) 1987; 512-521.
21. Uchio E, Takeuchi S, Itoh N, *et al*.: Clinical and epidemiological features of acute follicular conjunctivitis with special reference to that caused by herpes simplex virus type 1. Br J Ophthalmol 2000; 84(9): 968-972.
22. Azari AA and Barney NP: Conjunctivitis: A systematic review of diagnosis and treatment. JAMA 2013; 310(16): 1721-1729
23. Ravindran RD, Venkatesh R, Chang DF, Sengupta S, Gyatsho J, Talwar B: Incidence of post cataract endophthalmitis at Aravind Eye Hospital: Outcomes of more than 42,000 consecutive cases using standardized sterilization and prophylaxis protocols. J Cataract Refract Surg 2009; 35: 62936.
24. Sharma S, Gopalakrishnan S, Aasuri MK, Garg P and Rao GN: Trends in contact lens associated microbial keratitis in southern India. Ophthalmology 2003; 110: 13843.
25. Sharma S and Hemalatha S: DNA Fingerprinting Profile and Quality Control Standardization of Folklore Medicinal Plant *Exacum lawii*. Pharmacog J 2017; 9(1): 73-82. (In press)
    Kajaria DK, Gangwar M, Kumar D, Sharma AK, Tilak R, Nath G, *et al*.: Evaluation of antimicrobial activity and bronchodialator effect of a polyherbal drug-Shrishadi. Asian Pacific J Trop Biomed 2012; 2(11): 905-909.
26. Bhandari DK, Nath G, Ray AB and Tewari PV: Anti-microbial activity of crude extracts from *Berberis asiatica* stem bark. Pharmaceutical Biology 2000; 38(4): 254-257.
27. Aladag H, Ercisli S, Yesil DZ, Gormez A and Yesil M: Antifungal activity of green tea leaves (*Camellia sinensis* L.) sampled in different harvest time. Pharmacog Magaz 2009; 5: 437-440.
28. Wiegand I, Hilpert K and Hancock RE: Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nature Prot 2008; 3: 163-175.
29. National Committee for Clinical Laboratory Standards NCCLS. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standards- NCCLS document M7-A5. NCCLS. Wayne, PA, USA, Edition 5th, 2000.
30. Sharma AK, Gangwar M, Tilak R, Nath G, Kumar Sinha AS, Tripathi YB and Kumar D: Phytochemical characterization, antimicrobial activity and reducing potential of seed oil, latex, machine oil and presscake of *Jatropha curcas*. Avicenna J Phytomed 2016; 6(4): 366-375.
31. Koutsaviti A, Milenković M, Tzakou O: Antimicrobial activity of the essential oil of Greek endemic Stachys-spruneri and its main component, isoabienol. Nat Prod Commun 2011; 6: 277-80.
32. Stefanello MÉ, Pascoal AC and Salvador MJ: Essential oils from neotropical Myrtaceae: chemical diversity and biological properties. Chem Biodivers 2011; 8: 73-94.
33. Hassan MM, Oyewale AO, Amupitan JO, Abduallahi MS and Okonkwo EM: Preliminary phytochemical and anti-bacterial investigation of crude extracts of the root bark of *Detarium microcarpum*. J Chem Society Nig 2004: 29: 26-29.
34. Ostrovskii VA, Trifonov RE and Popova EA: Medicinal chemistry of tetrazoles. Russian Chemical Bulletin, International Edition. 2012; 61(4): 768-780.
35. Abirami P, Gomathinayagam M and Panneerselvam R: Preliminary study on the antimicrobial activity of *Enicostemma littorale* using different solvents. Asian Pacific journal of tropical medicine 2012; 5(7): 552-5.
36. Jesus JA, Lago JH, Laurenti MD, Yamamoto ES and Passero LF: Antimicrobial activity of oleanolic and ursolic acids: an update. Evidence-Based Complementary and Alternative Medicine. 2015; 2015.
37. Kumarsamy Y, NaharL, Cox PJ, Jaspars M and Sarker SD: Bioactivity of secoiridoid glycosides from *Centaurium erythraea*. Phytomedicine. 2003; 10: 344-347.

How to cite this article:
Sharma S, Nath G and Hemalatha S: Antimicrobial activity of *Exacum lawii* extracts and volatile oil against ocular infection. Int J Pharm Sci Res 2018; 9(6): 2266-72. doi: 10.13040/IJPSR.0975-8232.9(6).2266-72.