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Isolation and evaluation of the antimicrobial activity of *Sonchus asper, Tephrosia purpurea* and *Leucas indica* essential oils

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Abstract
In the present study essential oil of three herbs (*Sonchus asper*, *Tephrosia purpurea* and *Leucas indica*) was extracted and characterized by Gas chromatography. Characterization results showed the presence of several antimicrobial components in the essential oil of *Sonchus asper* such as dibutyl phthalate which is well known for its antibacterial efficacy. Antimicrobial efficacies of extracted essential oils were evaluated against five different pathogens i.e. *Pseudomonas aeruginosa* MTCC 2474, *Staphylococcus aureus* MTCC 1144, *Escherichia coli* MTCC 40, *Klebsiella pneumoniae* MTCC 4030 and *Streptococcus pyogenes* MTCC 442. The essential oil of *Sonchus asper* gave maximum zone of inhibition against *Staphylococcus aureus* MTCC 1144 which is known to be the main causal organism of wound infection whereas *Tephrosia purpurea* and *Leucas indica* showed the least efficacy against all the test organisms.

Keywords: essential oil; extraction; characterization; antimicrobial efficacy; gas chromatography

Introduction
Herbal medicines are known to be the basis of the treatment in India which are utilized as a cure for various diseases as well as physiological conditions in a traditional practice. There is a huge chance for the discoveries of new medications from the products of these herbs either as pure compounds or as standardized extracts (Cos *et al*., 2006) [4]. Medicinal components of plants play an important role in conventional medicines. Nowadays, a considerable amount of medicines have been developed which are relatively active against a huge number of ailments (Azwanida, 2015) [1]. Considering a huge variety of purposes medicinal plants extract and essential oil has been utilized traditionally. As far as their potential concerned they have been tested for several activities such as antimicrobial activity which has formed the basis of many applications, food preservation and processing, pharmaceuticals and natural therapies (Kamazeri *et al*., 2012) [11].
Essential oils also play a major role in many areas such as c olours, fragrance products, lotions, soaps, detergents, etc.
Most of the time these are volatile and tend to solubilize freely in ether, alcohol and mineral oils (Hernandez *et al*., 2000) [10]. Mainly hydro-distillation method is considered for the extraction of essential oils using Clevenger -type – apparatus which was proposed by Clevenger, (1928) [3]. The apparatus consists of separator, distillation flask, condenser and heating mantle. Material having essential oil is placed in distillation flask attached with separator. On applying temperature oil distillate with steam condenses and falls on the graduated tubes of the separator. Oil is automatically separated in the separator and water flows back to the boiling flask (Clevenger, 1928) [3]. In the present study extractions of essential oils of *Sonchus asper*, *Leucas indica* and *Tephrosia purpurea* were carried out and their antimicrobial potential was evaluated against some ubiquitous pathogens actively participates in wound infections. All the three herbs are being utilized in the ethnic framework for their therapeutic qualities mainly in the skin and wound infections (Deshpande *et al*., 2003 [6]; Navaneethan *et al*., 2011 [15]; Upadhyay *et al*., 2013) [20].

2. Materials and Methods
2.1. Collection of medicinal plant
Leucas indica collected from the town Devprayag in Tehri Garhwal district of Uttarakhand, India. The herb was collected at the end of August. *Sonchus asper* was collected from Kankhal in Haridwar district, Uttarakhand, India. The herb was collected in early December. *Tephrosia purpurea* was collected from the bare land beside Gurukula Kangri Vishwavidyalaya, Haridwar, Uttarakhand, India. All the herbs were authenticated from Botanical Survey of India, Dehradun having accession numbers *Sonchus asper* (L.) Hill (116606), *Leucas indica* (L.) Sm. (116608) and *Tephrosia purpurea* (L.) Pers. (116607).

2.2. Extraction of essential oil
Collected plant materials were shade dried for 15 days and coarsely powdered. Extraction of the essential oils were carried out by hydro-distillation method using Clevenger type apparatus which is also recommended by pharmacopoeia. 250 g of powdered material with distilled water was immersed in a round bottom flask attached to the assembly of Clevenger type apparatus and placed on the heating mantle at the boiling point of water (Samadi *et al*., 2017) [18]. After the extraction oil was allowed to stand for the short interval of time and then separated by *n*- Hexane using separating funnel. Separated suspensions with *n*-Hexane were purified by vaporizing *n*- Hexane at 60ºC on the hot plate.

2.3. Characterization of the oil sample
Essential oils of the given plant materials were characterized by GC-MS technique from CIMAP Lucknow, India using Perkin Elmer SQ8 C MS and Clarus 680 GC with EI ionization 70 eV which was fitted with Elite 5 MS column having dimensions of 30 m × 0.25 mm × 0.25 µm. Mass to

charge ratio (m/z) was 50 to 450 amu with scan time 0.8 sec and interscan delay was 0.01 sec.
Injector temperature was 250° C and the temperature for the oven for the analysis was programmed from 60–240°C at 3°C/min with an initial hold of 6 min. Helium was used as a carrier gas at a flow rate of 1 ml/min with a split ratio of 1:100. MS data were analyzed by Wiley and NIST Library search which was further compared with standard published data.

2.4. Antimicrobial activity

Antimicrobial activity of essential oils was evaluated against five different standard pathogens i.e. *Pseudomonas aeruginosa* MTCC 2474, *Staphylococcus aureus* MTCC 1144, *Escherichia coli* MTCC 40, *Klebsiella pneumoniae* MTCC 4030 and *Streptococcus pyogenes* MTCC 442 by agar well diffusion method. The bacterial inoculums were prepared by the adjustment of turbidity according to McFarland standards 0.5. The quantity of 100 µl of each bacterial inoculum were spread onto the solidified Muller Hilton agar medium plates and a well of 6 mm diameter was punched by sterile cork borer at the centre of each plate. 50 µl of essential oil sample was mixed with 950 µl of DMSO to make final concentration 1 ml and 45 µl of essential oil samples were poured into the wells. All the plates were tested in triplicates and incubated at 37˚ C for 24 hours. Antibiotic disks of cefazolin (30mcg), streptomycin (10 mcg) and ampicillin (10 mcg) were used as a positive control.

3. Results

Percentage yield of oil

The percentage (%) yield was calculated using the following equation:

$$\text{Percentage yield of oil} = \frac{\text{Volume of oil}}{\text{Weight of plant material}} \times 100$$

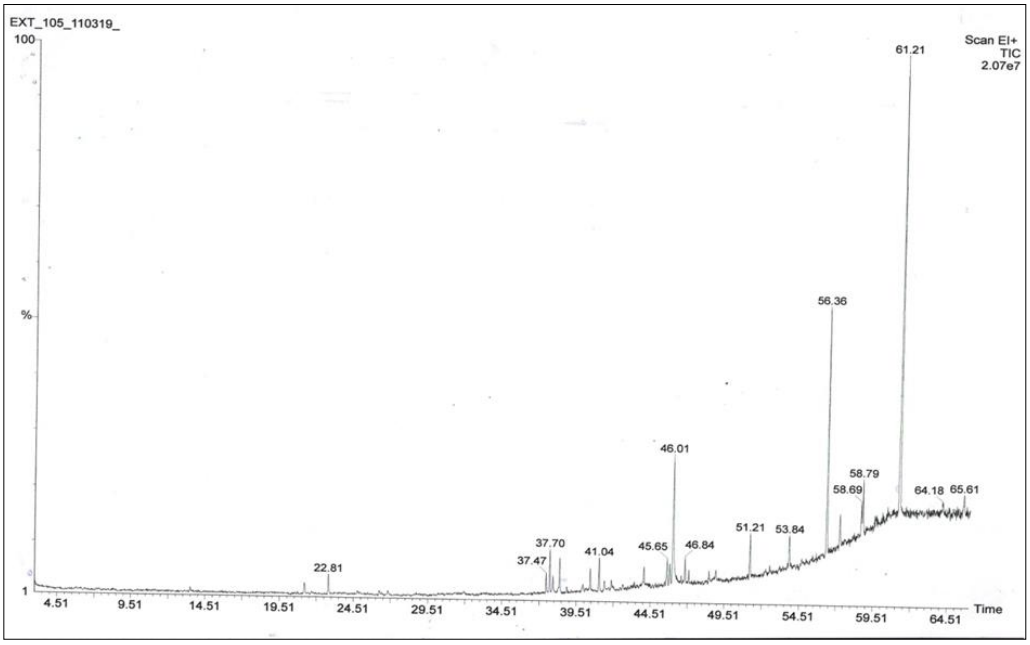

Fig 1: Chromatogram of GCMS result for *Sonchus asper* essential oil.

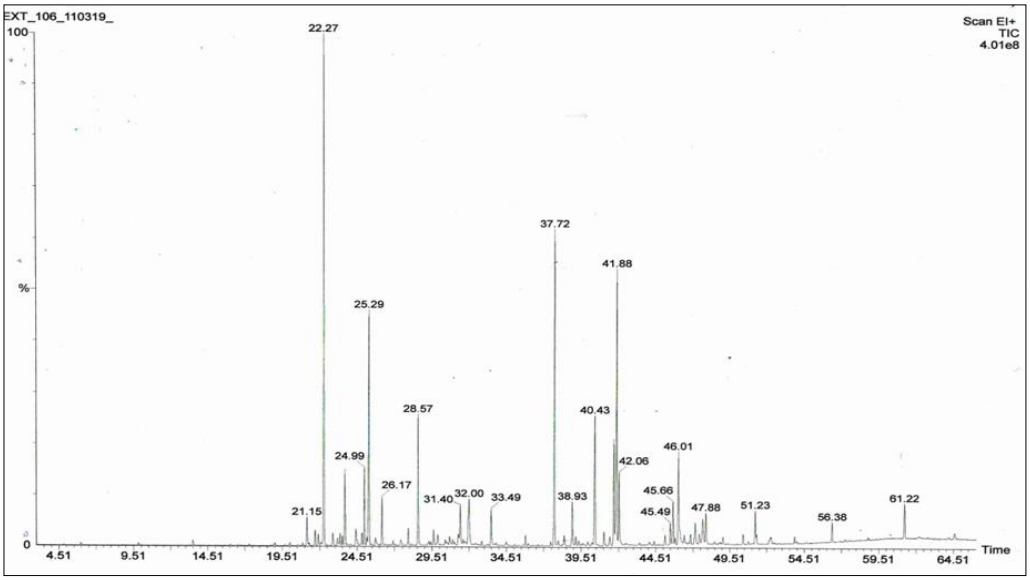

Fig 2: Chromatogram of GCMS result for *Leucas indica* essential oil.

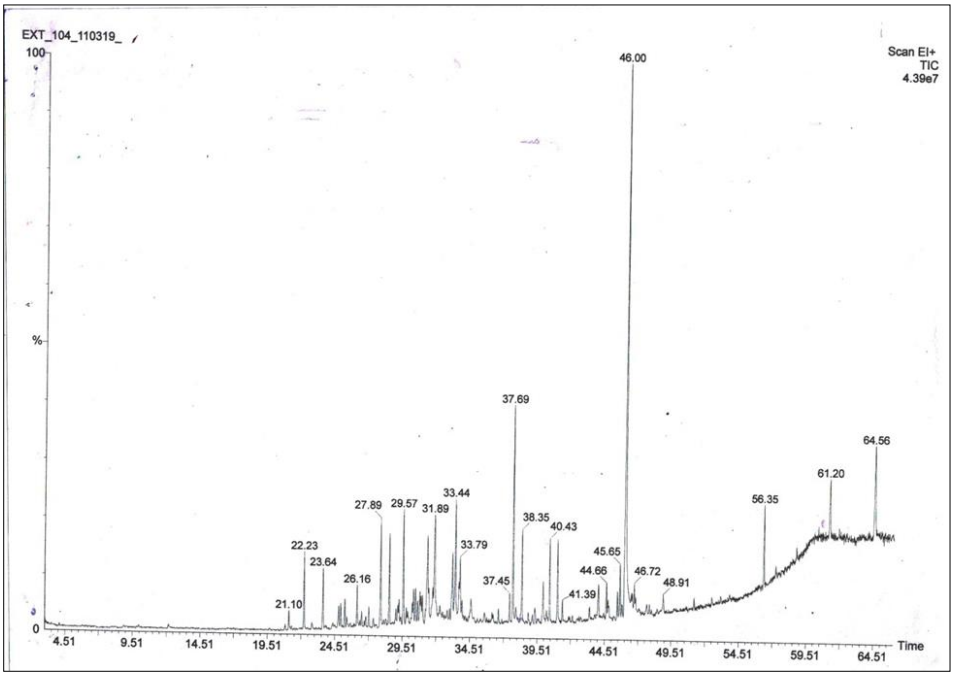

Fig 3: Chromatogram of GCMS result for *Tephrosia purpurea* essential oil.

Antimicrobial activity

The essential oil of *Sonchus asper* gave promising result against all the tested pathogens. It showed best antimicrobial potential against *Staphylococcus aureus* MTCC 1144 (15.00±0.00 mm) followed by *P. aeruginosa* and *S. pyogenes* (14.00±0.00 mm), *E. coli* (12.66±0.33 mm) and *K. pneumoniae* (12.00±0.00 mm) (Figure 4). Essential oil of *Tephrosia purpurea* showed maximum zone of inhibition against *E. coli* (09.66±0.33 mm) followed by *K. pneumoniae* (09.00±0.00 mm), *P. aeruginosa* (08.66±0.66 mm), *S. pyogenes* (08.033±0.33 mm) and *S. aureus* (07.00±0.00 mm) (Figure 5). Maximum zone of inhibition for *Leucas indica* essential oil was observed against *P. aeruginosa* (11.00±0.57 mm) followed by *S. aureus* (07.66±0.33 mm), *E. coli* and *S. pyogenes* (07.00±0.00 mm). There was no activity observed against *K. pneumoniae* for the essential oil of *Leucas indica* (Figure 6). As a positive control commonly used antibiotics i.e. ampicillin, cefazolin and streptomycin that are utilized in wound infection were used.

Among antibiotics maximum zone of inhibition for ampicillin was recorded against *E. coli* (20±0.57 mm) followed by *P. aeruginosa* (15±0 mm), *K. pneumoniae* (11.66±0.33 mm), *S. aureus* (09.66±0.33 mm) and *S. pyogenes* (7.66± 0.33). Cefazolin gave maximum antimicrobial potential against *E. coli* (24±0.57 mm) followed by *P. aeruginosa* (12±0 mm) *S. aureus* (10.00±0.57 mm), *K. pneumoniae* (09.66±0.33 mm) and *S. pyogenes* (7±0 mm). The maximum zone of inhibition for streptomycin was observed against *P. aeruginosa* (36±0 mm) followed by *E. coli* (25.66±0.57), *S. aureus* (14.33±0.57 mm) and *S. pyogenes* (14±0 mm). There was no activity against *K. pneumoniae*. All the values of the zone of inhibition include the diameter of cork borer and disks used for the antibacterial activity. The comparative study on the antibacterial efficacy of all the three extracted essential oils revealed that *Sonchus asper* has maximum zone of inhibition against all the tested pathogens as compared to essential oils of *Tephrosia purpurea* and *Leucas indica* (Figure 7).

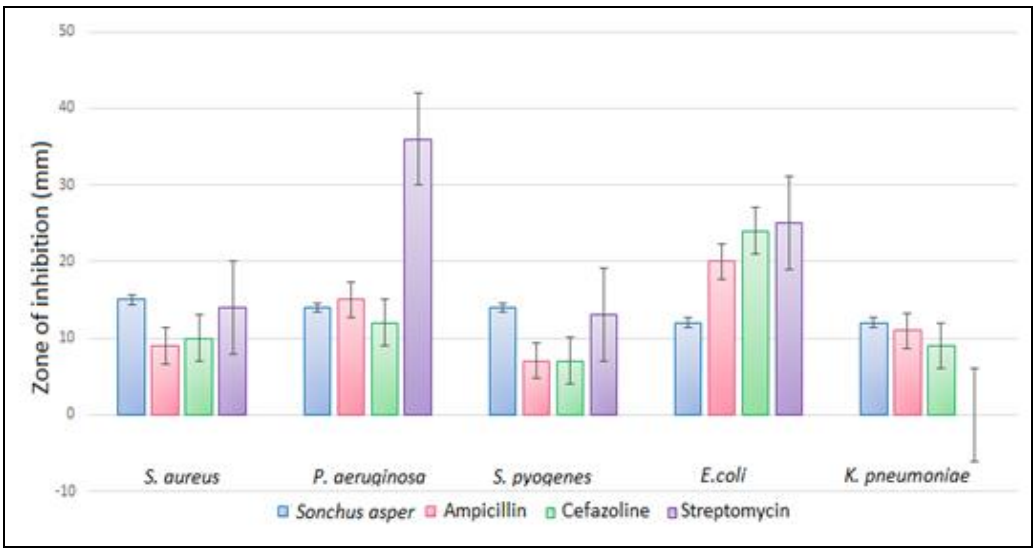

Fig 4: Graphical representation of the antimicrobial potential of *Sonchus asper* essential oil and comparison with standard drugs.

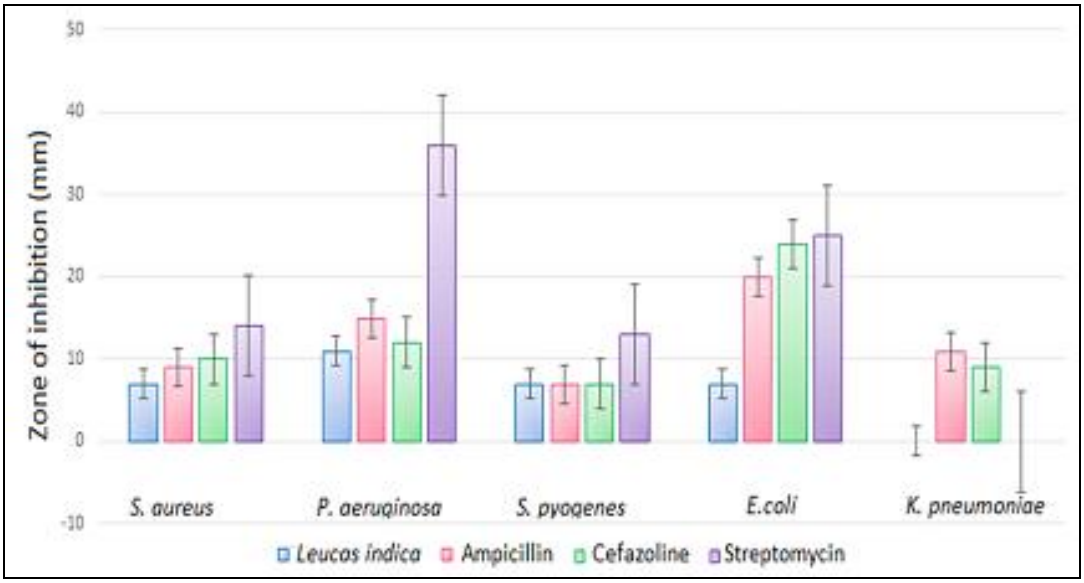

Fig 5: Graphical representation of the antimicrobial potential of *Leucas indica* essential oil and comparison with standard drugs.

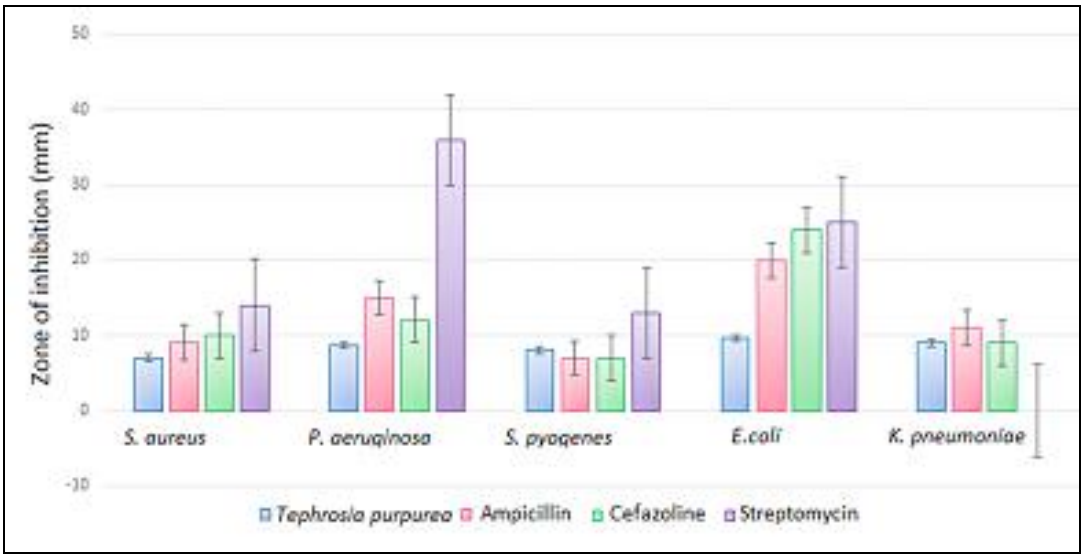

Fig 6: Graphical representation of the antimicrobial potential of *Tephrosia purpurea* essential oil and comparison with standard drugs.

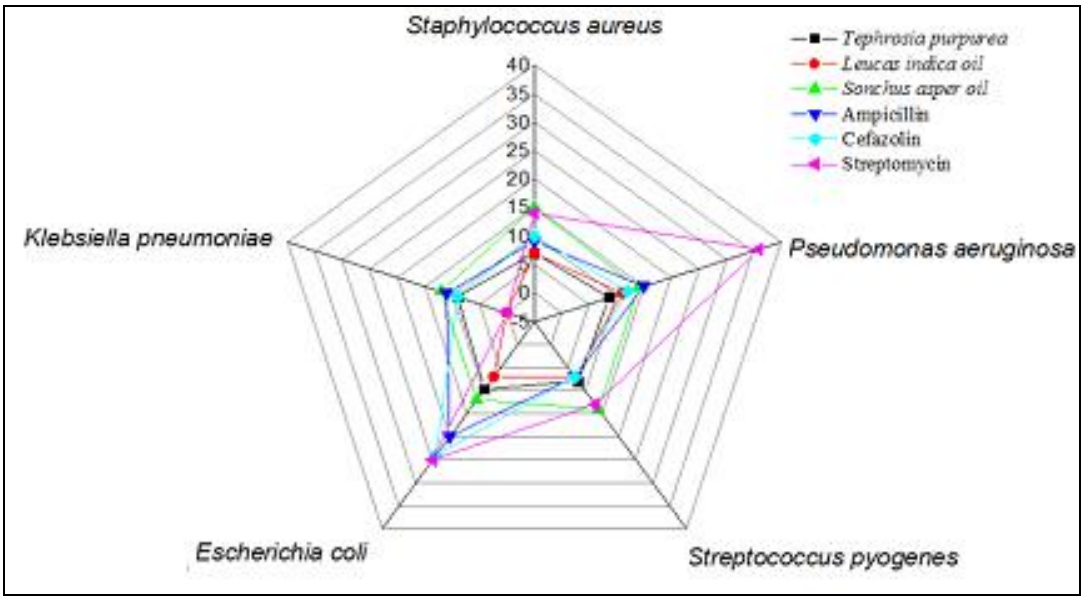

Fig 7: Comparative study of antimicrobial potential of *Tephrosia purpurea, Leucas indica* and *Sonchus asper* essential oils.

4. Discussion

There are certain factors i.e. composition of essential oils, functional groups present in their bioactive compounds etc. are important and necessary for the antimicrobial efficacy of the particular essential oil (Chouhan *et al*., 2017) [2]. The bioactive compounds present in the essential oils may adhere to the cell surface that leads to penetrating the lipid bilayer causing death of the cell (Lv *et al*., 2011 [14]; El Kolli *et al*., 2016) [7]. GCMS data of the essential oil of *Sonchus asper* reveals the presence of prominent antimicrobial bioactive compounds such as Dibutyl phthalate, beta-Citronellol, *n*-Octadecane etc. Similarly, essential oil of *Tephrosia purpurea* also contains few antimicrobial compounds which includes Di isobutyl phthalate, Caryophyllene, Methyl palmitate etc. The essential of *Leucas indica* didn't showed such compounds except Caryophyllene that are known for their antimicrobial activity. Previously antimicrobial potential of Dibutyl phthalate has been reported which was isolated from *Streptomyces nasri* (El Naggar, 1997) [8]. Similarly in a study by Katiwora, *et al*., (2012), Dibutyl phthalate was isolated from the *Ipomea carnea* and tested against three Gram's negative bacteria *Klebsiella pneumoniae* (ATCC 33495), *Proteus mirabilis* (ATCC 12453) and *Pseudomonas aeruginosa* (ATCC 10662). They noticed significant activity against all the tested organisms among which *Pseudomonas aeruginosa* was most susceptible. The same compound was isolated from *Begonia malabarica* by Shobi and Vishwanathan, (2018). On testing its antimicrobial efficacy, they found that at the dose of 100 mg/ml it showed considerable potential against *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Shigella flexneri*, *Vibrio cholera* and *Pseudomonas aeruginosa*. Lopez-Romero, *et al*., (2015) [13] performed the antibacterial activity of citronellol against *Escherichia coli* and *Staphylococcus* they found that the changes in hydrophobicity, surface and membrane integrity were due to the leakage of K^+ ions which may result in the cell lysis. While Ghannadi, *et al*, (2012) [9] performed antibacterial activity of essential oil of *Pelargonium graveolens* L' Her against *Listeria monocytogenes* (PTCC 1297), *Salmonella enteritidis* (PTCC 1091), *Pseudomonas aeruginosa* (PTCC 1330), *Staphylococcus aureus* (PTCC1112) and *Bacillus subtilis* (PTCC 1023).

Except *Listeria monocytogenes* all the bacterial strains were susceptible, they found the activity of essential oil was due to the presence of beta-Citronellol.

Rouis –Soussi, *et al*., (2014) [7] performed antibacterial activity of essential oil of *Allium nigrum*, they found that the tested bacterial strains were sensitive against the essential oil due to the presence of certain aliphatic compounds such as *n*-octadecane. Yaylı *et al*., (2009) [21] reported the considerable antimicrobial activity of *Centaurea appendicigera* essential oil. They found the β-caryophyllene as a major component of the essential oil of *Centaurea appendicigera*. Pinto *et al*., (2016) [16] isolated fatty acid methyl esters from the vegetable oils among which methyl palmitate was found to be most abundant and gave considerable antimicrobial efficacy. In a study by Davoodbasha *et al*., (2017) [5] similar results were obtained while extracting fatty acid methyl esters from microalgae *Scenedesmus intermedius.* On performing antibacterial activity they found that the fatty acids were active against *E. coli and P. aeruginosa*.

Conclusion

The essential oil of the *Sonchus asper* gave considerable results against all the tested pathogens. GCMS result reveals the presence of some antibacterial components such as dibutyl phthalate, beta-Citronellol, *n*-Octadecane etc. Although essential oil of *Leucas indica* and *Tephrosia purpurea* has also given antimicrobial efficacy but not up to the extent that they could be considered for any formulations. On conclusive remark, we can say that the essential oil of *Sonchus asper* can be utilized as an antibacterial agent which can apply externally to the skin during microbial infection of the wound.

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