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Essential Oil Extraction, Characterization and Antimicrobial Study of *Blumea laciniata* DC from Konkan Region

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ABSTRACT: The present study of Essential Oil of *Blumea laciniata* DC was designed to evaluate essential oil extraction, phytochemical composition and Anti-Microbial Study of essential oil from *Blumea laciniata* DC collected from Dapoli Tehsil in Konkan Region. The Essential Oil was extracted by methods using like Hydro distillation using Clevenger's type Apparatus, Steam Distillation and Solvent Extraction. Characterization of essential oil was done by IR, GC-MS and Anti-Microbial Activity. Total 34 compounds were identified using Gas Chromatography with Mass Spectroscopy. The main components of essential oil are Caryophyllene Oxide (7.63%), Tau. Muurolol (5.79%), Armoadendrene Oxide (4.00%), Oleic Acid (3.50%), 1-Heptatriacotanol, 1(+)-Ascrobic Acid 2,6-dihexadecanoate, etc. IR confirms the identification of components. Chemical Investigation was done to determine its molecular formula and structure.

Keywords: *Blumea laciniata* DC; Essential Oil Extraction Method; IR; GC-MS and Anti Microbial Activity.

INTRODUCTION: Asteraceae or Compositae commonly referred as the aster, daisy, composite[1] or sunflower family. It is very large and wide spread family of flowering plants (Angiospermae).[2 & 3] The family currently has 32,913 accepted species names in 1,911 genera and 13 subfamilies.[4] Most members of Astreaceae are herbaceous but a significant number also shurbs, vines and trees. The species of these families are generally observed from the Polar Regions to the tropics. Colonizing a wide variety of habitats. It is most common in the arid and semiarid regions of subtropical and lower temperate latitude.[5] Asteraceae is an economically important family providing product such as cooking oils, lettuce, sunflower, seeds, artichokes, sweetening agents, coffee substituents and herbal teas.[6] The ability to utilize oxygen has provided humans with the benefit of metabolizing carbohydrates, fats and proteins for energy, however it does not come without a cost. A contradiction in metabolism is that, while the vast majority of complex life on earth requires oxygen for its existence. Oxygen is highly reactive. Atom that is capable of becoming part of potentially damaging molecules commonly called "Free Radical". Free radical are capable of attacking the healthy cells of the body causing them to lose their structure and function.[7 & 8] Cell damage caused by free radicals leads to major contributor to aging and degenerative diseases such as cancer, cardiovascular diseases, immune system decline and brain dysfunction.[7 & 9] Overall free radicals have been implicated in the pathogenesis of at least 50 diseases. [7, 10 &11]

Almost all studies on essential oil research focus on their extraction, chemical composition and wide application in the food and cosmetic industries and traditional medicines.[12 & 13] Therefore it is often believed that essential oils are completely safe as they are natural in origin. Essential Oils are rich blend of highly concentrated, volatile and fat soluble in nature. Therefore mainly differ from the water soluble whole herb extracts used in herbal medicines.[14] The toxicity of essential oils can also be entirely different to that of the herb as they are lipophilic in nature and hence can pass across the membranes very efficiently.[15] As these properties are beneficial for their medicinal effects, this may also lead to their toxicity. Some of the major areas of concern about essential oil hazards include allergic contact dermatitis, photosensitization, neurotoxicity, carcinogenicity.[16] Hence it is very important to study interaction of essential oils and their constituents in vivo to know their efficiency as well as toxicity. As the fragrance of essential oils are complicated and difficult to characterize, there are very few reports available about their in vivo interactions in body fluids.[17, 18 & 19] Therefore it is more important to find out

right methodology for identification of constituents of essential oil from easily available aromatic plants.

Asteraceae family consists of genera *Blumea* which is vast in its species. From these *Blumea* species, *Blumea laciniata* DC is collected first time from the konkon Region. To find out phytochemical constituents present in this plant with the help of Anti Microbial Study of essential oil obtained by different extraction processes.

Antimicrobial resistance is a major global problem with resistant strains of Staphylococcus aureus[18] and Pseudomonas Aeruginosa[19] and other micro organisms being responsible for much morbidity and mortality.

Medicianl Plants have the ability to inhibit the growth of wide range of pathogenic micro organisms due to presence of essential oil.[20] Essential oils are natural, volatile liquid, complex compound characterized by strong odor, rarely colored, soluble in lipids and organic solvents. It could be synthesized by all plant organs i.e. buds, flowers, leaves, stems, twings, seeds, fruits, roots, wood or bark and are stored in secretary cells, cavities, canals, epidermic cells or grandular trichomes.[21]

The Konkon region is rich biodiversity. *Blumea laciniata* DC is easily available species in paddy fields in Konkan Region. In India no one did work on *Blumea laciniata* DC. This is the first research article which is contributing in an identification of phytochemical constituent present in *Blumea laciniata* DC. Essential Oil generally contains terpens, sesquiterpens, alkaloids, flavonoids, etc. Essential Oil of *Blumea laciniata* DC is consisting of total 34 components which are identified by GC-MS. This is showing how much this species is showing variety in constituents. Antimicrobial study of essential oil of this species shows very efficient against Staphylococcus Aureus and Psedomonas Aeruginosa bacteria's. It means that essential oil of *Blumea laciniata* DC inhibit the growth of these microorganisms very efficiently. Essential Oil of this species is an alternative to the diseases caused by micro organisms.

Most importantly *Blumea laciniata* DC is easily available and people around here throwing away this species but they don't know the medicinal use of species. This research article is showcasing the medicinal use of *Blumea laciniata* DC in konkan region.

About *Blumea laciniata* DC: *Blumea laciniata* DC is very common Rabbi Weed in india. *Blumea laciniata* DC is an annual herb having strong odor like turpentine. In An Indian System of Traditional Medicines i.e. Ayurveda, *Blumea laciniata* DC is used as bitter, astringent, acrid, thermogenic, errhine, anti-inflammatory, styptic, ophthalmic, digestive, anthelmintic, liver tonic, expectorant, febrifuge, antipyretic, diuretic, deobstruant and stimulant. Taxonomy of Genus *Blumea laciniata* DC is as follows:

Kingdom: Plantea
Order: Asterales
Family: Asteraceae
Tribe: Astereae
Genus: *Blumea*

Blumea laciniata DC belongs to genus *Blumea* and family Astreaceae. *Blumea laciniata* DC is commonly called as a spiny leaved blumea. *Blumea laciniata* DC an annual erect herb with a slender whitish hairy stem, which is often forked. The branches are spreading or prostrate. Alternately arranged obovate leaves, 5-5.5 X 1.5-2 cm, have spinous toothed margin, and a spiny tip. Leaf stalks are up to 1 cm long. Leaves on branches 1 X 0.5 cm, nearly stalk less, densely white woolly. Yellowish flower heads, to 6 mm across, on long peduncles, arise in leaf axilsy, either solitary or in corymb like cymes. Flowering period of *Blumea laciniata* DC starts from January.

MATERIAL AND METHODS:

Plant Material: The entire plant including leaves, stem, aerial part, flowers of *Blumea laciniata* DC were collected from the paddy fields of Talsure in Dapoli region, Maharashtra, India between the months of December to May. After collection of *Blumea laciniata* DC particular species was submitted to The Botanical Survey of India Western Region Pune Maharashtra for identification and certification. They have certified and identified this species as *Blumea laciniata* DC.

Essential oil Extraction: Essential oil Extraction of *Blumea laciniata* DC was carried out methods using like Hydro distillation by using Clevenger's apparatus, Steam Distillation and Solvent Extraction Method.

Essential Oil Extraction by Hydro Distillation with Clevenger's Apparatus Method: Essential Oil Extraction of *Blumea laciniata* DC was carried out with Hydro distillation by using Clevenger's Apparatus Method. The fresh plant material including aerial part, stem, leaves and flowers gets chopped into small pieces. 50 gm of fresh plant material was subjected to hydro distillation using Clevenger type apparatus of capacity 1 liter. Only 70 ml of water was added just to wet the fresh plant material. The mixture was heated on heating mental at 85ºC. The distillation was continued for about 3 hours. As the essential oil obtained

was in very less quantity i.e. 0.2 ml that's why I have to carried out the hydro distillation process number of times till I get the desired quantity for characterization of essential oil. After obtaining desired quantity it was dried over by anhydrous sodium sulphate and stored in sealed vials in refrigerator until analysis.

Essential Oil Extraction by Steam Distillation Method: Essential Oil Extraction of *Blumea laciniata* DC was carried out with Hydro distillation by using Clevenger's Apparatus Method. The fresh plant material including aerial part, stem, leaves and flowers gets chopped into small pieces. 50 gm of fresh plant material was subjected to Steam distillation using Steam Distillation apparatus of capacity 1 liter. 400 ml of water was added to develop vapors to pass from the fresh plant material. The water was heated on heating mental at 85ºC. The distillation was continued for about 3 hours. As the essential oil obtained was in quantity i.e. 0.25 ml that's why I have to carry out the Steam distillation process number of times till I get the desired quantity for characterization of essential oil. After obtaining desired quantity it was dried over by anhydrous sodium sulphate and stored in sealed vials in refrigerator until analysis.

Essential Oil Extraction by Solvent Extraction Method: Essential Oil Extraction of *Blumea laciniata* DC was carried out using Solvent Extraction Method. The fresh plant material including aerial part, stem, leaves and flowers gets chopped into small pieces. Approximately 50 gm of fresh plant material was subjected to solvent extraction method. This plant material was kept in vessel to be socked in solvent named as ethyl alcohol (100ml) for about 24 hours. After 24 hours ethyl alcohol gets evaporated in water bath to get the desired quantity of essential oil of *Blumea laciniata* DC. 50 gm fresh plant material gives approximately 0.25 ml of essential oil of *Blumea laciniata* DC.

Characterization of Essential Oil: Essential Oil characterization of *Blumea laciniata* DC was done by the techniques like Gas Chromatography with Mass Spectroscopy, Infrared Spectroscopy and Nuclear Magnetic Resonance Spectroscopy.

RESULTS AND DISCUSSION:

Infrared Spectroscopy: Infrared Spectroscopy (Figure 1) gives information on the vibrational and rotational modes of motion of a molecule and hence an important technique for identification and characterization of a functional group. The infrared spectrum of an organic compound a unique fingerprint which is readily distinguished from the absorption patterns of all other compounds. An IR analysis was accomplished using Bruker, 3000 Hyperion Microscope with vertex 80 FTIR system equipped with focal plane array of 128 X 128 and ranges from 4000 – 900 cm^{-1}. It does have single point detector ranging from 7500 – 450 cm^{-1}. It is having analysis area 128 X 128 in 2D format on the sample plane 300 X 300 µm. This instrument is having spatial resolution with 15 times objective is 2.7 µm, temperature controlled sample stage and spectral resolution of FTIR is 0.2 cm^{-1}. (Figure 1)

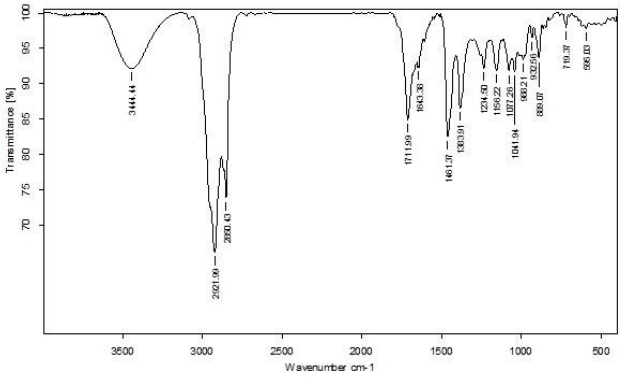

Figure 1: IR Spectra of Essential Oil.

Gas Chromatography: Initially GC was used for development of chromatographic method for the selected plant essential oil. The GC analysis was accomplished using Shimadzu GCMS-QP 2010 Ultra gas chromatograph equipped with FID and Rtx®-5 MS capillary column (0.25mm X 30m X 0.25 µm film thicknesses). Following temperature program was optimized for analysis. (Table 1)

Table 1: The optimized temperature program for column oven.

Minutes	Rate	Final Temperature (0^0 C)	Hold Time (minute)
0	-----	70.0	0.00
1	20.00	200.0	3.00
2	25.00	300.0	2.00
3	0.00	0.0	0.00
Total Program Time			15.50 minutes

Injector temperature was 240ºC while detector temperature was 225ºC. Helium was used as a carrier gas, at a flow rate 1.53 cm3/ min. Split ratio was 1:25.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis: The GC method was then transferred to GC-MS with slight modifications for identification of various phytoconstituents of selected plant essential oil. The oil was analyzed by Shimadzu GCMS-QP

2010 Ultra system. The system was equipped with fused silica Rtx-1 Sil MS silarylene capillary column with dimensions 30m X 0.25mm X 0.25µm. Helium (0.93 ml/min) was used as a carrier gas. The program used for GC oven temperature was 1 minute isothermal at 50ºC, followed by 50-220ºC at a rate of 50^0C/min, then held at 220ºC for 1minute, followed by 220 - 260ºC at a rate of 200ºC/min, then again held at 260ºC for 15 minutes. The injection port temperature was 266ºC. The ionization of sample components was performed in the E.I. mode (70eV). The Linear Retention Indices (LRI) for all the compounds was determined by co-injection of the sample with a solution containing the homologous series of C8-C29 *n*-alkanes. Individual constituents were identified by referring to compounds known in the literature data and also by comparing their mass spectra with known compounds and NIST Mass Spectral Library (NIST 05). (Figure 2)

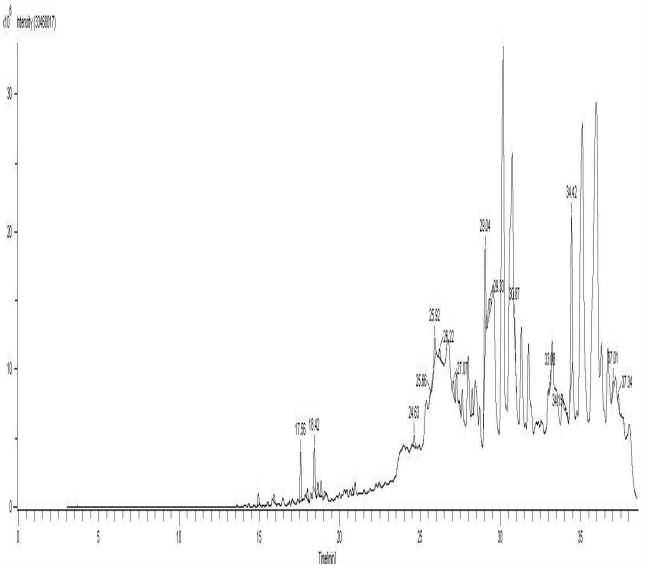

Figure 2: GC-MS of Essential Oil.

Table 2: Chemical Components of Essential Oil of *Blumea laciniata* DC.

Relative Retention Indices	Relative concentration of components in Area Percentage (%)	Name of Compound
14.94	1.78	Bicyclo[7.2.0] unde-4-ene-4,11,11- trimethyl-8-methylene[1R-(1R*,4Z,9S)]
15.90	2.74	1H-Cyclopenta(1,3) cyclopropa (1,2) benzene, octahydro-7- methyl-3-methylene-4-(1-methylethyl)- [3aS-(3aS-(3aα, 3bβ, 4β,7a,7aS*)]
17.56	7.63	Caryophyllene Oxide
18.00	2.33	Caryophyllene Oxide
18.42	5.79	Tau-muurolol
18.64	1.18	Aromadendrene Oxide I
20.80	1.16	9,12-Octadecadienoic acid (Z,Z)
24.63	1.70	1(+) Ascorbic Acid 2,6-dihexadecanoate
25.66	2.02	Oleic Acid
26.40	1.78	Trans-13-octadecenoic acid
27.07	1.61	Oleic Acid
27.44	1.15	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy
28.55	3.61	1-Heptatriacotanol
29.04	2.48	8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)
30.73	1.30	1H-Naphthol (2,1-b) pyran-3-ethynyldodecahydro-3,4a,7,7,10a-pentamethyl-[3R-(4αβ,10aβ,10bα]
30.87	1.32	Tert-hexadecanethiol
31.30	2.30	1b,4a-epoxy, 2H-Cyclopenta(3,4) Cyclopropa (8,9) Cycloundec (1,2) Oxiren-5-(1aH)- One,2,7,9,10-tetrakis (acetyloxy) decahydro-3,6,8,8,10a-pentamethyl
31.74	3.90	1-Heptatriacotanol
33.08	3.90	1-Heptatriacotanol
33.22	2.46	9-Octadecenoic acid (z), 2-hydroxy-1-(hydroxymethyl) ethyl ester
34.15	6.81	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol (3β,5Z,7E)
34.42	3.94	2,6,10,14,18,22-Tetracosahexaene-2,6,10,15,19,23-hexamethyl(a,E)
35.09	1.57	Cyclopenta(a,d) Cycloocten-5-one,1,2,3,3a,4,5,6,8,9,9a,10,10 a-dodecahydro-7-(1-methylethyl)-1,9a-dimethyl-1,9a-dimethyl-4-methylene
35.96	1.04	Podocarp-7en-3-one, 13R-methyl-13-vinyl
36.27	2.27	2-Pentanoic Acid, 5-decahydro-5,5,8a-trimethyl-2-methylene-1-naphthalenyl-3-methyl [1S-(1α(E),4aβ, 8aα)]
36.38	2.27	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy
37.01	2.56	Cyclopenta(a,d) Cycloocten-5-one,1,2,3,3a,4,5,6,8,9,9a,10,10 a-dodecahydro-7-(1-methylethyl)-1,9a-dimethyl-1,9a-dimethyl-4-methylene
37.34	2.09	Tert-Hexadecanethiol

Antimicrobial Activity: Anti-Microbial Activity (Table 3) of Essential Oil from *Blumea laciniata* DC was done against five microorganisms. Out of these five microorganisms four microorganisms were Gram negative and remaining one were Gram Positive Bacteria. Agar Cup Method was used to evaluate the results of Anti-Microbial Activity. In this test streptomycin solution having concentration 25 µg/ml was used as a standard against extracted essential oil. Volume for Anti-Microbial Activity Standard Solution and Essential Oil 20 µl/well was used. Anti-microbial activity of Essential oil of *Blumea laciniata* DC was checked by making two different concentrations like 1:4 (Essential Oil : Acetone) diluted concentration of essential oil, undiluted (crude) concentration of essential oil. Concentration of these two samples of essential oil was checked against standard named as streptomycin (25 µg/disc). Results of Inhibition Zones obtained from both were as follows:

Table 3: Inhibition Zones (mm).

Cultures	Gram Character	Diameter of Inhibition Zone (mm)		
		Pure Extract of Essential Oil	1:4 (Extract : Acetone)	Streptomycin (25 µg/disc)
Escherichia Coli	Gram Negative	1	0	15
Pseudomonas Aeruginosa		0	0	9
Proteus Vulgaris		0	0	0
Salmonella Typhi		3	0	20
Staphylococcus Aureus	Gram Positive	5	0	16

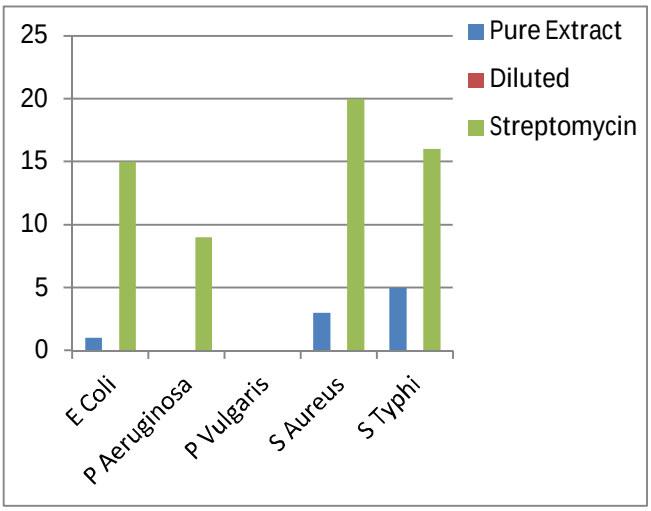

Figure 3: Graph of Inhibition Zones in mm against Cultures of Micro organisms.

DISCUSSION: The hydro distillation using Clevenger's apparatus in which 100 gm chopped plant material yields 0.10% of brown color essential oil with a sweet smell. In case of steam distillation yield obtained was more than hydro distillation i.e. 0.19%. The oil sample was analyzed by Gas Chromatography with Mass Spectroscopy (GC-MS) and Infrared Spectroscopy (IR) the components were identified on the basis of their Retention Index Values and by comparison of their mass spectra with those reported in literature. The GC-MS analysis of *Blumea laciniata* DC essential oil shows total 34 components were identified, shown in Table 1. There was presence of Sesquiterpene alcohol, lactones, monoterpenes, diterpenes, triterpenes, terpenoids. The main components of essential oil The main components of essential oil are Caryophyllene Oxide (7.63%), Tau. Muurolol (5.79%), Armoadendrene Oxide (4.00%), Oleic Acid (3.50%), 1-Heptatriacotanol, 1(+)-Ascrobic Acid 2,6-dihexadecanoate, etc. Fourier Transform Infrared Spectroscopy (FT-IR) shows presence of functional groups which were identified in Gas Chromatography with Mass Spectroscopy (GC-MS). FTIR shows various stretching such as 3423.95 cm^{-1} indicates alcohol stretching, 2925 cm^{-1} indicates C-H stretching, 2726 cm^{-1} indicates C-H stretching, 1714-1646 cm^{-1} indicates fingerprint region in which compounds like ketones, esters, etc. were present. The initial spectra exhibit three main peaks in this range: 2954.47, 2923.56 and 2854.60 cm^{-1}. The 2954.47 and 2923.56 cm can be assigned to the anti-symmetric stretching modes of the CH_3 and CH_2 groups. The 2923.56 cm is due to corresponding symmetric stretching. The behavior of Essential Oil in IR Spectra indicates a decrease in number of CH_2 groups. There is a dissociation of chains of fatty acid. Further support of this hypothesis negative contribution about finger print region in IR spectra where C=O stretching is observed. The spectra suggest that these groups are increasing during aging. This could interpret that there is possible esterification reaction takes place. Some of the major compounds such as Bicyclo[7.2.0] unde-4-ene-4,11,11- trimethyl-8-methylene [1R-(1R*,4Z,9S)] is commonly known as cis-caryophyllene having large pharmacological effects such as it is anti microbial agent, antipyretic agent, analgesic etc. Tau. Muurolol which is higher percentage in essential oil is used as anti-microbial agent, anti-pyretic. The principal use of oleic acid is as a component in many foods, in the form of its triglycerides. It is a component of the normal human diet as a part of animal fats and vegetable oils. Oleic acid as its sodium salt is a major component of soap as an emulsifying agent. It is also used as an emollient.

1(+)- Ascrobic Acid 2,6-dihexadecanoate is one of the fatty acid present in essential oil. It is used as anti-microbial agent.

Anti-microbial activity of Essential oil is performed against 5 microorganisms Escherichia Coli, Pseudomonas Aeruginosa, Salmonella Typhi, Protus Vulgaris, Salmonella Typhi, Staphylococcus Aureus. Out of these five cultures of microorganisms four were gram negative i.e. Escherichia Coli, Pseudomonas Aeruginosa, Protus Vulgaris, Salmonella Typhi and one was gram positive i.e. Staphylococcus Aureus. An Anti-Microbial Activity was done against the standard solution of streptomycin having concentration of 25 µg/ml. Out of these 5 cultures of Micro Organisms Essential oil showed very good results of inhibition zones for Escherichia Coli, Salmonella Typhi and Staphylococcus Aureus. The most important that Inhibition zones of essential oil were more than the standard streptomycin solution as well as acetone solution both having volume of 20 µl/ml. for these two microorganisms. Staphylococcus Aureus causes range of illness from minor skin infections, such as pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome and abscesses, to life threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia and sepsis. Pseudomonas Aeruginosa causes a diseases like urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections and a variety of systemic infections, particularly in patients with severe burns and in cancer and AIDS patients who are immunosuppressed. As an essential oil of *Blumea laciniata* DC shows very good results than standard streptomycin solution against these microorganisms i.e. Pseudomonas Aeruginosa, Staphylococcus Aureus. This is proposed that essential oil of *Blumea laciniata* DC restrict the growth of diseases caused by Pseudomonas Aeruginosa, Staphylococcus Aureus.

Therefore, our results revealed the importance of plant extracts when associated with antibiotics, to control resistant bacteria, which are becoming a threat to human health. Furthermore, in a few cases, these plant extracts were active against antibiotic resistant bacteria under very low concentration, thus minimizing the possible toxic effects.

CONCLUSION: Analytical Method has been used for the identification of phyto constituents of essential oil of *Blumea laciniata* DC from Astreascea family. This methodology includes GC-MS, FT-IR for the identification of volatile phyto constituents. Mass Spectroscopy has been used for the exact mass measurement with identification of phyto constituents. Anti-Microbial Activity has been used to evaluate the therapeutic use of this essential oil from *Blumea laciniata* DC against standard streptomycin solution. Specifically yield given by dry plant material is much more than yield given by the fresh plant material. This is the most important observation by experimentation. Essential Oil of *Blumea laciniata* DC has great potential as anti-microbial compounds against these microorganisms. Thus they can be used in the treatment of infectious diseases caused by resistant microbes. Most importantly *Blumea laciniata* DC is easily available in konkan region but this is the first research article showcasing the importance of this species. Essential Oil of *Blumea laciniata* DC from Konkan Region contains various phtyto components such as Caryophyllene Oxide (7.63%), Tau. Muurolol (5.79%), Armoadendrene Oxide (4.00%), Oleic Acid (3.50%), 1-Heptatriacotanol, 1(+)- Ascrobic Acid 2,6-dihexadecanoate and it is a plant of pharmaceutical use. Essential oil of *Blumea laciniata* DC had been showing medicinal as well as pharmacological use. It may be proposed that this oil may be further use in pharmaceutical or in cosmetic industries.

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