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Editorial

Currently G-20 summit is going to take place on 9-10 September 2023 **in** India is stressing on Make in India, Skill India, Startup India, Invest India, Digital India and Atmnirbhar Bharat like initiatives. All this needs research and UJPAH is partnering in its humble way through participating in research initiatives of young scientists.

I am happy to share that you can find tremendous growth in the research initiatives in science fraternity as I mentioned same 9 years ago that research papers published in India on herbs are 10 times less than China. Now this has improved tremendously and India is on 3rd position in the world. I congratulate the young and senior science fraternity people for the hard work in this direction, also there is an improvement noticed in IPR of Indian products.

Therefore this issue is based on "AMR and Indian herbs as promising source of Antimicrobial Compounds" and millets which can be another source of health products. Millets are called "The Future Super Food for the World". Our Prime Minister Shree Narendra Modi sponsored the proposal for International Year of Millets (IYM) 2023 which was accepted by United Nation General Assembly (UNGA).

We should find out the plants rich in Vitamin D like Alfalfa (*Medicago sativa*), Wild mushrooms, like oyster, button, chanterelle, and shiitake mushrooms, Microalgae,), *Wattle seed, Tasmanian mountain pepper, and leaves of Solanaceae family plants like Tomato, Potato, Egg plant,* waxy-leaf nightshade (*Solanum glaucophyllum & Solanumm elongena) et c*are additional herbs that may work to promote vitamin D levels. Second deficiency is of Iron and Vitamin A especially low hemoglobin level in ladies. Iron deficiency can be treated by using Iron rich herbs like Spinach, Fenugreek, Beetroot, Dandelion and Nettle leaves (Bichhubooti). In Vitamin A deficiency we may take herbs like Dill seeds, Coriander seeds, Basil leaves and Cayenne pepper.

I hope the deliberations of this seminar will be very useful for all participants and my sincere thanks to all who have contributed their valuable research for publication and make this issue a success. I would like to thank the entire editorial, advisory members and seminar committees for their untiring efforts in bringing out this issue. I offer my best wishes to all those scientist, research scholars, students and teachers who contributed for bringing out this issue and also express my sincere gratitude to all Board members who made this issue a memorable for scientific fraternity of Uttarakhand and the country as a whole.

Dr. S. Farooq
Chief Editor

Contents

Identification and quantification of cinnamic acid derivatives in *Cichorium intybus* seed and its extract by High-Performance Liquid Chromatography with Diode-Array Detector (HPLC-DAD) and Electrospray Ionization Mass Spectrophotometry (LC-MS/MS)

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Abstract- A sensitive method coupling high-performance liquid chromatography (HPLC) with diode –array detector (DAD) and electrospray ionization mass spectrometry (MS) was optimized for separation, identification, and quantification of cinnamic acid derivatives in *Cichorium intybus* seed and its extract. Cinnamic acid derivatives such as chlorogenic acid, caffeic acid and Chicoric acid were quantified using respective standards. Apart from 4-o-Caffeoylqunic acid, other cinnamic acid derivative such as 3-o-caffeoylquinic acid was also identified and quantified by UV and MS/MS spectra and calculated as total caffeoylquinic acids using 4-o-caffeoylqunic acid as standard in the seed and its extract. Other cinnamic acid derivatives such as 1,3-dicaffeoylqunic acid, 1,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, 3, 4-dicaffeoylquinic acid, dicaffeoylquinic acid-1 and dicaffeoylquinic acid-2 (two unknown) were identified and quantified by UV and MS spectra and calculated as total dicaffeoylquinic acids using chlorogenic acid standard in the seed and its extract. The total cinnamic acids were quantified by calculating the sum of chlorogenic acid, caffeic acid, chicoric acid, total caffeoylquinic acids(4-o-caffeoylquinic acid and 3-o-caffeoylqunic acid) and total dicaffeoyl-quinic acids(1,3-dicaffeoylqunic acid, 1,4-dicaffeoylquinic acid, 3,5-dicaffeoyl-quinic acid, 3, 4-dicaffeoylquinic acid, dicaffeoylquinic acid-1 and dicaffeoyl-quinic acid-2). The Phytochemical screening of *C. intybus* seed and its extract revealed that this plant is a rich source of cinnamic acid derivatives so, these markers (cinnamic acid derivatives) can used for routine quality control of *Cichorium intybus* seed and its extract.

Keywords- *Cichorium intybus* L, Cinnamic acid, HPLC-DAD, LC-MS/MS

Introduction

Cichorium intybus L. is commonly known as chicory is an erect woody perennial herb with about1 m height and

fleshy taproot up to 75 cm in length with large basal leaves[1-3]. It belongs to a family of Asteraceae. Ancient Egyptians grew chicory as a medicinal plant, coffee substitute, and vegetable crop. It was used occasionally for many years for animal forage. This plant is used for diabetics due to its negligible impact on blood sugar [4]. Chicory can tolerate extreme temperatures during both vegetative and reproductive growth stages [1]. All parts of the plant exudates milky latex. In Europe, Asia and Africa the *Cichorium intybus* issued as a medicinally important plant. Even though it has been used for many years, there is no monograph is available in the European Pharmacopoeia or in any official Pharmacopoeia of a European Union member state [5]. However, due to its widespread distribution, different parts of the plant have been used globally in traditional medicines[7]. There are many medicinally important compounds such as alkaloids, inulin, sesquiterpene lactones, coumarins, vitamins, chlorophyll pigments, unsaturated sterol, flavonoids, saponins and tannins are reported in this plant[9-11]. Insulin's a naturally occurring polysaccharides produced industrially from chicory[12-15]. The insulin belongs to class of dietary fibers. In plants, Insulin is used as a means of storing energy and is typically found in roots or rhizomes.

C. intybus has been traditionally used for the treatment of fever, diarrhea, jaundice and gallstones [16-21]. The studies revealed that *C. intybus* possesses anti-hepatotoxic,anti-diabetic [22-24] anti-bacterial [25,26,27], anti-inflammatory[28,29], hyperglycemic and anti-ulcerogenic activities. The *C. intybus* seed contain phenolic constituent's mainly cinnamic acid derivatives such as chlorogenic acid, caffeic acid, Chicoric acid, 4-caffeoylquinic acid, other caffeoylquinic acids and dicaffeoylquinic acids [30, 34]. There are few methods are available for the identification of phenolic constituents in this plant. A high-performance liquid chromatography coupled with mass spectrometry in tandem mode method was established for the analysis of various cinnamic acid derivatives. The seeds were first subjected to hydro alcoholic extraction with 2:1ethanol and water followed by evaporation and spray drying. Reversed-phase liquid chromatography coupled by an electrospray (ES) interface to an ion trap mass spectrophotometry (MS) was used for the separation of the various cinnamic acid derivatives. The identification and quantification of cinnamic acid derivatives such as chlorogenic acid, caffeic acid, chicoric acid, caffeoylquinic acids and dicaffeoylquinic acids has been achieved using this chromatographic technique.

Material and methods

Plant materials- *Cichorium intybus* seeds were collected from Bengaluru market, Karnataka, India. The seeds were identified and authenticated at the R & D Center, Himalaya Wellness Company, Bengaluru, and Karnataka, India.

Chemicals and Reagents- All the organic solvents were of LC-MS and HPLC grade and were purchased from Thermo Fisher. The LC-MS grade water was purchased from J. T. Baker. Acetic acid was procured from Rankem, Acetonitrile from Fisher scientific and

methanol from Finer. The standard chlorogenic acid,4-o-caffeoylquinic acid, caffeic acid and chicoric acid were purchased from Sigma-Aldrich. Purified water used for the preparation of mobile phase is from Milli-Q water purification system (Millipore, Pure lab, Classic, ELGA).Filtration membranes of 0.45 µm cellulose acetate/cellulose nitrate mixed esters were purchased from Millipore.

Sample preparation

Powdering of the material- The dried samples of *C. intybus seeds* were ground using a rotary grinder, sieved through 25 mesh sieves and stored in airtight HDPE container at room temperature. The analytical variations can be minimized using the powdered sample which is used throughout the study for further study.

Extraction procedure- Accurately weighed about 100 g of air-dried powdered material into a 1000 ml round bottom flask and extracted by refluxing on water bath at 85^0C to 90^0C using 500 mL ethanol and water in the ratio (2:1).The process was repeated for two more times with of 300 mL ethanol and water in the ratio 2:1.The hydro alcoholic extract was filtered, concentrated and dried at 105 ± 2^0C. The crude extract obtained after drying was used for further analysis.

Standard solutions- Standard solution of chlorogenic acid, caffeic acid, chicoric acid and4-o-caffeoyl quinic acid was prepared at a concentration of 0.05 mg/ml with methanol.

Test solution- The powdered seed and hydro alcoholic extract was extracted with methanol by refluxing on water bath at 80^0C at a concentration of 20 mg/ml and 10 mg/ml respectively. The extract was filtered through a 0.45 µm syringe filter and 10 µl was injected into the HPLC-PDA-ESI/MS system for analysis.

HPLC and LC-ESI-MS analysis

The chromatographic separation was achieved by a high-performance liquid chromatography with photo diode array detector. The HPLC used was Shimadzu Prominence-i equipped with a photo diode array detector, SIL-20ACHT auto sampler, DGU-20A5 degasser, LC-20AD pump, CBM-20 A system controller, CTO-10 ASVP oven and LC solutions software. Many trials were conducted for the separation of the phyto-compounds. The best chromatographic condition was achieved using 0.2 % v/v acetic acid in water (mobile phase A) and 0.2 % acetic acid in acetonitrile and purified water in the ratio of 1:1 (mobile phase B). The linear gradient elution was performed with the following ratio of mobile phase B 15%, 0 minutes, 35%, 20 minutes, 45%, 25-35minutes, 15%, 45 minutes at a flow rate of 1 mL/minute with a column oven temperature of 40°C.

Mass spectrometric method- Mass scans were acquired on API 2000 (Applied biosystem/MDS SCIEX, Canada) mass spectrometer coupled with ESI (Electron spray ionization) source with HPLC chromatographic system. Analyst 1.5 version software was used for the batch acquisition and data processing. The ion trap has been operated in data-independent, full scan (100-1000 m/z), zoom scan, and MS^n mode to obtain fragment ion m/z with a collision energy of 35% and an isolation

with 3 m/z. Data dependent acquisition, where user-specified criteria are applied to select the ion of interest for subsequent fragmentation, are among the most useful approaches employed to identify unknown compounds by MS. Using this approach single stage MS provides the molecular mass that can be obtained by tandem MS analysis via the fragmentation pathway. When greater discrimination was required additional targeted MS^2 experiments were performed on selected pseudo molecular ions.

The MS parameters were optimized with 0.05 mg/mL standard solutions. The sample was run, and intensity was checked in both positive and negative ionization mode. Subsequently, good intense response was observed in the negative mode and other parameters like declustering potential (DP) -60v, nebulizing gas (GS1 and GS2)55 and 65psi, curtain gas (CUR) 30 psi, focusing potential (FP) -400 v, Entrance potential (EP) -10 v and source temperature (TEM) 420°C and Collision energy (CE) for fragmentation of precursor to product ions were optimized through multiple runs using LC in order to reach the each of the most intense precursor to product ion. The negative and positive parameters of the ion mode ESI source has achieved by flow injection analysis, using chlorogenic acid at a concentration of 0.05mg/ml.

For MS analysis the negative ion mode of ESI was selected because it provided extensive structure information for most phenolic acids present in *Cichorium intybus*. In addition to detection of the deprotonated molecular ions, collision induced dissociation was performed in the MS^2 and MS^3, and the resulting product ions were used as fingerprints of each component.

Quantification of total caffeoylquinic acids (3-o-caffeoylquinic acid and 4-o-caffeoylquinic acid) has achieved using standard of 4-o-caffeoylquinicacid. The dicaffeoylquinic acids (1,3-dicaffeoyl quinic acid, 1,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, 3,4-dicaffeoylquinic acid, dicaffeoylquinic acid-1 and dicaffeoylquinic acid-2) were quantified using chlorogenic acid as standard. Identification and quantification of chlorogenic acid, caffeic acid and chicoric acid has been calculated using respective standard. Identification was carried out by UV spectra, retention time with authenticated standards followed by confirmation from mass spectra of the corresponding peaks.

Validation- The developed method was validated as per the International Conference on Harmonization (ICH) guidelines. The validation parameters include specificity, system suitability, precision, accuracy, range and robustness.

System Suitability- System suitability has been executed by injecting six injections of standard chlorogenic acid. The parameters such as theoretical plates (N), peak asymmetry factor (As), % relative standard deviation (% RSD) and retention time were recorded.

Specificity- The specificity study has done by injecting blank, standard and the sample solution to find out the interference at the analyte retention time (RT) of chlorogenic acid with diluents

peak. The peak purity profile was established using diode array detector.

Precision- The precision of the method was performed by injecting three replicates of three different concentrations at 50%, 100% and 150% of working concentration of 10mg/mL sample solution on the same day(intraday precession) and different day (inter day precession)and the % RSD was calculated.

Linearity- Linearity was performed by preparing five different concentrations of the sample ranging from 50% to 150% of working concentration of 10 mg/mL sample solution (100%).

Accuracy- It can be defined as exactness of an analytical method or the closeness of results. The accuracy was derived once the precision, linearity and specificity had been established (option 'c' as per the ICH guideline (Q2 R1 validation). Since accuracy was derived from precision, linearity and specificity,

acceptance criteria should comply as mentioned for the precision, linearity and specificity.

Robustness- Reliability of the method was done with respect to deliberate variations in method parameters like variation in the flow of the mobile phase (± 0.1 ml/min), variation in temperature (38°C to 42° C) and change in column by injecting duplicate injections of single preparation.

Results and discussion

The Hydro alcoholic extract obtained from *C. intybus* seed was submitted to HPLC analysis. The chromatographic method was developed to explore various phytoconstituents from *C. intybus* seed and its extract. Different methods have been designed by changing column, mobile phase, organic modifier, and with different concentration of samples to separate the different derivatives of cinnamic acid from the seed powder and its extract. The best separation was achieved using 0.2 % v/v acetic acid in water (mobile phase A) and 0.2 % acetic acid in acetonitrile and purified water in the ratio of 2:1 (mobile phase B). **Figure-1**.

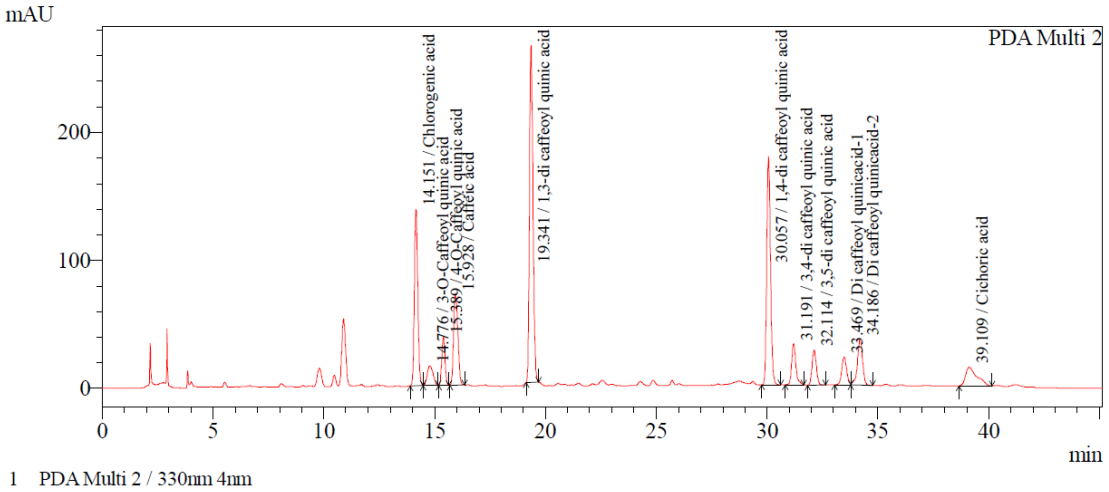

Figure-1 HPLC chromatogram of *C. Intybus* extract at 330 nm

The optimized HPLC-PDA method was validated for the simultaneous analysis of cinnamic acid derivatives such as chlorogenic acid, caffeic acid and chicoric acid using respective standard and 3-o-caffeoylquinic acid and 4-o-caffeoylquinic acid as total caffeoylquinic acids using 4-o-caffeoylquinic acid and 1,3 di caffeoylquinic acid, 1,4 di caffeoylquinic acid, 3,4 di caffeoylquinic acid, 3, 5 di caffeoylquinic acid and two di caffeoylquinic acids-1 & 2 (unknown di caffeoylquinic acids) quantified as total dicaffeoylquinic acids using chlorogenic acid as standard. **Table-1**.

Table-1 Identification and quantification of Cinnamic acid derivatives in *Cichorium intybus* seed and extract.

	Chlorogenic acid (% w/w)	Caffeic acid (% w/w)	Chicoric acid (% w/w)	Total caffeoylquinic acids (% w/w)	Total di caffeoylquinic acids (% w/w)	Total cinnamic acid derivatives (% w/w)
C. Intybus seed	0.09	0.01	0.062	0.10	0.53	1.38
C. Intybus extract	0.40	0.11	0.21	0.69	1.64	3.22

Mass analysis and identification

In this study,1 1 compounds were identified and characterized by UV-Spectra and MS/MS data and the standard solutions of chlorogenic acid, caffeic acid, chicoric acid and 4-o-caffeoylquinicacid were prepared and injected to compare the retention times.

The HPLC-DAD chromatograms and total ion chromatograms (TIC) in the negative mode of the extracts of *Cichorium intybus* are shown in Figure-2. The application of MS for the analysis of Cinnamic acid derivatives has increased by soft ionization techniques. These compounds are polar nonvolatile and thermally liable.

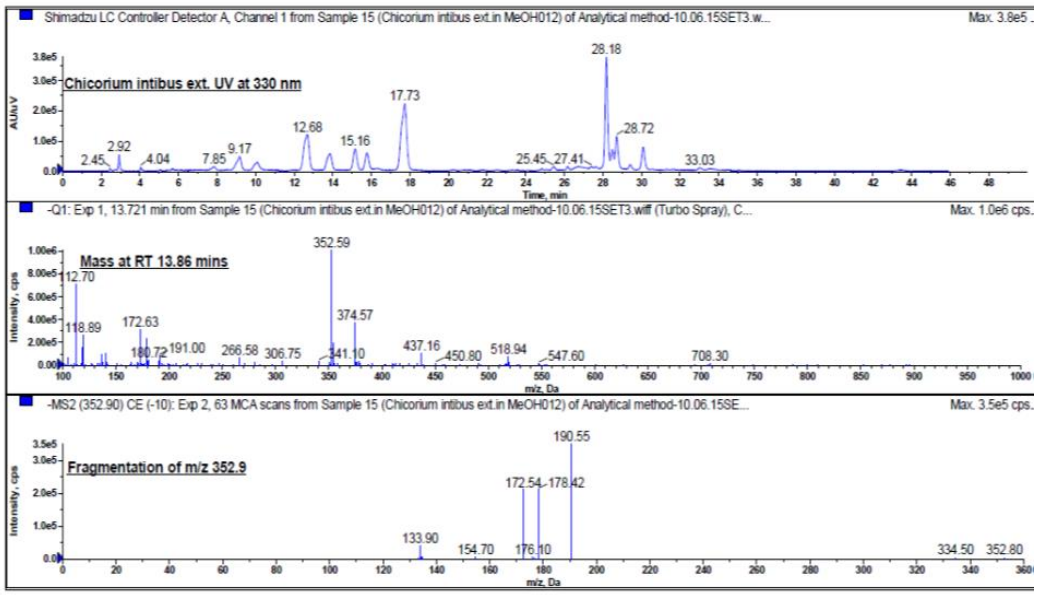

Figure-2 LC-MS/MS data of *C. Intybus* extract

The UV and Mass spectra of phyto-compounds confirmed that they are all mono-caffeoyl quinic acid (CQA) isomers. The mono-CQA has an ion at m/z 191 as the base peak [M-H caffeoyl] _ when the acyl group is linked to the 3-OH or 5-OH position, and an ion at m/z 173 when the acyl group is attached to the 4-OH position. The 3-CQA and 4-CQA were differentiated based on the relative intensity of m/z 179 $[M-H]^-$ quinic acid, which is more significant for 3-OH compounds. Based on the retention times, UV–Vis spectra, and MS2 fragments with the standard, a 3-CQA and 4-CQA were identified.

Dicaffeoylquinic acids eluted later in the chromatogram, gave $[M-H]^-$at m/z 515, and MS2 fragment at m/z 353, corresponding to the loss of a caffeoyl moiety $[M-H]^-$162as the base peak. The presence and intensity of MS2 secondary fragments confirms the dicaffeoylquinic acid isomers.The presence of Ion with m/z 299 (intensity 35%) and the less intense fragments at m/z 255 and 203 were unique characteristic of 4-acyl dicaffeoylquinic acid.

Hydro alcoholic extract of *C. intybus* yielded 1,3-dicaffoylquinic acid, 1,4-dicaffoylquinic acid, 3,4-dicaffoyl quinic acid, 3,5-dicaffoyl quinic acid, dicaffeoylquinic acid-1 and dicaffeoyl-quinic acid-2 with parent ion at m/z 515. **Table-2.**

Table-2 LC-MS/MS data of chromatographic peaks

Peak no.	Compound name	UV λmax (nm)	MS $[M-H]^-$ (*m/z*)	MS/MS (*m/z*), (Abundance)
1	Caffeic acid	330nm	179	MS^2[179]: 135 (100)
2	3-O-Caffeoylquinic acid	330nm	353	MS^2[353]: 191 (100), 179 (50), 135 (5)
3	4-O-Caffeoylquinic acid	330nm	353	MS^2[353]: 191 (15), 179 (60), 173 (100)
4	Chicoric acid	330nm	473	MS^2[473]: 311 (100), 293 (20), 179 (45), 149 (30)
5	Chlorogenic acid	330nm	353	MS^2[353]: 191 (100), 179 (2)
6	1,3 dicaffeoylquinic acid	330nm	515	MS^2[515]: 353 (100), 335 (30) MS^3[353]: 191 (100), 179 (50)
7	1,4 dicaffeoylquinic acid	330nm	515	MS^2[515]: 353 (100), 299 (35), 317 (28), 255 (10), 203 (5) MS^3[353]: 191 (24), 173 (100)
8	3,4 dicaffeoylquinic acid	330nm	515	MS2[515]: 353 (100), 299 (25), 255 (18), 203 (8)
9	3,5 dicaffeoylquinic acid	330nm	515	MS^2[515]: 353 (100), 191 (18) MS^3[353]: 191 (100), 179 (50), 173 /2), 135 (10)
10	Dicaffeoylquinicacid-1	330nm	515	MS^2[515]: 353 (100)
11	Dicaffeoylquinicacid-2	330nm	515	MS^2[515]: 353 (100)

Conclusion

The lifestyle diseases and chronic health issues are a major health problem worldwide. The health care management demand to deliver an optimal environment for better treatment and to meet the need of human population. In this context, identification of phytomarkers with therapeutic property are very essential for the ailment of many chronic diseases. The quality of health care can be improved by exploring the medicinal plants which were used in the traditional medicine.

The present study was aimed to identify the maximum phyto constituents in *C. intybus* seed and its extract and establish the method to quantify the same for routine quality control of seed. Many trials were conducted to achieve the well separation of phyto markers. The chromatographic method developed for HPLC, and LC-MS/MS is able to resolve all the phenolic compounds present in *C. intybus* seeds and its extract. There are about eleven cinnamic acid derivatives such as chlorogenic acid, caffeic acid,chicoric acid,3-o-caffeoylquinic acid, 4-o-caffeoylquinic acid, 1,3di caffeoylquinic acid, 1,4 di caffeoylquinic acid, 3,4 di caffeoylquinic acid,3, 5 di caffeoylquinic acid, di caffeoylquinic acid-1 and di caffeoylquinic acid-2 were identified and quantified. The chromatographic method developed for HPLC and LC-MS/MS for the identification and quantification of cinnamic acid derivatives in the seeds of *C. intybus* seed and its extract has shown similar chromatographic profile. The developed method confirms in terms of precision, accuracy, linearity, specificity, and robustness. It was observed that there were no relevant variations attributable to the nature of the detected fragments or their relative intensities were observed.

The present study has attained the chromatographic identification and quantification of different cinnamic acid derivatives in *C.intybus* seeds and its extract. This method can be used for the standardization of *C. intybus* seed and to find out the authenticity and quality of crude drug. This analytical data is an important tool for the development of herbal raw material as a medicinal ingredient and will enable to open a window in search of phyto markers as bio active compounds.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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Cell based models for validation of safety of cosmetic products and ingredients

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Abstract- The use of cosmetic products has grown many folds in recent years. The consumer and regulatory bodies are more concerned about the hazardous outcome of these products on skin health. The development of alternative and effective safety assessment methodologies to avoid animal testing is an urgent need due to restrictions and ethical considerations set by various regulatory bodies such as OECD, COLIPA, and BIS. The new integrated approaches like *in-vitro* assays (Cell-based and Cell-free assays) are indispensable for assessing the safety of cosmetic products. *In-vitro* testing systems provide advantages over animal testing as they are more cost-effective, and less time-consuming. In this article, we present the details of some of the important *invitro* assays that are routinely used by different laboratories for validation of the safety of cosmetic ingredients.

Key words: Safety and toxicity, Skin irritation, Skin sensitization, Eye irritation, Phototoxicity, Chromosomal aberration.

Introduction

In today's highly competitive personal care and cosmetic product market, evidence that the product is safe and effective can mean the difference between getting noticed and getting lost in the crowd. The EU defines a cosmetic product as "any substance or preparation intended to be placed in contact with the various external parts of the human body (epidermis, hair system, nails, lips, and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odors and/ or protecting them or keeping them in good condition[1]. Personal care products may contain some ingredients which can potentially cause adverse reactions when applied locally or systemically. Regulatory authorities worldwide have made it mandatory to prove the safety of any products intended for human use. In earlier years various toxicology testing methods and guidelines were developed by different agencies across the globe and most of them involved wide use of different animals; for example, Draize test for eye irritation was done in rabbits, acute oral and reproductive toxicity is done in mice etc. In recent years, there was a large outcry led by organizations like PETA and Humane Society International against the widespread use of animals for cosmetic testing. In a historical decision, the European Cosmetic and Perfumery Association (Colipa) in its 7th Amendment to the Cosmetics Directive (2003/ /15/EC) introduces a progressive ban on animal testing and a marketing ban on cosmetic products and their ingredients that are tested on animals[2]. The

Government of India prohibited the testing of cosmetics on animals vide G.S.R. 346(E) in 2014 by inserting the following rule in the D & C Rules (Drugs and Cosmetics Act 1940 and Rules 1945), "148-C: Prohibition of testing of cosmetics on animals- No person shall use any animal for testing cosmetics"[3]. Further, it was specified that when there is a need to demonstrate the absence of adverse reaction, the manufacturer shall submit the safety data based on alternative non-animal test methods. In China where pre-market animal tests for cosmetic ingredients were previously mandatory, it's National Medical Product Administration (NMPA) has approved two new non-animal tests (for skin sensitization and eye irritation) for cosmetics ingredient testing.

Development of relevant in-vitro tests requires selecting appropriate endpoints and also analysis and appreciation of the key initiating events that occur in vivo in the progression of the toxic damage. In 1992 Colipa created the Steering Committee on Alternatives to Animal Testing (SCAAT) to coordinate the efforts of the cosmetics industry in the development, validation, and acceptance of alternatives to animal testing for evaluating the safety of products and ingredients[4]. In Europe, alternative testing methods developed by different laboratories are scientifically validated by the European Centre for the Validation of Alternative Methods (ECVAM), an official body appointed for this purpose by the European Union[5]. The ECVAM has proposed a list of validated cell-based in vitro models for predicting the safety and toxicity of cosmetic ingredients[6]. On an international level, Colipa works with the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) in the United States, as well as with partners from Canada and Japan[7]. OECD has developed Guidelines for the testing of chemicals which are a collection of the most relevant internationally agreed testing methods used by governments, industry, and independent laboratories to assess the safety of chemicals. They adhere to the concept of 3R's (Reduce, Replace, and Refinement) for animal usage and have validated protocols for in vitro genotoxicity, skin corrosion, skin absorption, phototoxicity, ocular severe irritation, and corrosion, as well as for screening potential endocrine disrupters. OECD countries and full adherents have agreed that a safety test carried out in accordance with the OECD Test Guidelines and Principles of Good Laboratory Practice in one OECD country must be accepted by other OECD countries for assessment purposes. In wake of a growing concerns of product safety amongst the consumers across the world and need for adopting alternative methods for animal testing, establishing in vitro assay methods to evaluate the safety of personal care products is of utmost importance for several laboratories. Several cosmetic laboratories, including ours, are progressively adopting various in vitro methodologies for ensuring the safety of personal care products before taking them to clinical studies and marketing. Different safety parameters such as skin and eye irritation/corrosion, phototoxicity, skin sensitization etc have been addressed in the array of tests that we have adapted according to our product portfolio. Different mammalian cell lines are used in accordance with the internationally validated and approved protocols (OECD guidelines) to establish the safety profile of personal care products. The in-vitro tests to check the adverse reactions such as skin irritation (OECD TG439), eye irritation (OECD TG491, 492), phototoxicity (OECD TG432), and skin sensitization (OECD TG442C&E) have been established in our laboratory.

In-vitro assays and models to assess the safety of cosmetic products

In-vitro assays and models are used to assess the toxicity and safety of test substances in

biological systems under artificial conditions without the involvement of animals. There are a variety of *in-vitro* assays developed to test the safety of cosmetic ingredients, and identification of hazardous ingredients. In the sections below, we discuss some of the routinely used cell based assays. **Figure-1** summarizes the types of the assay systems discussed in this article.

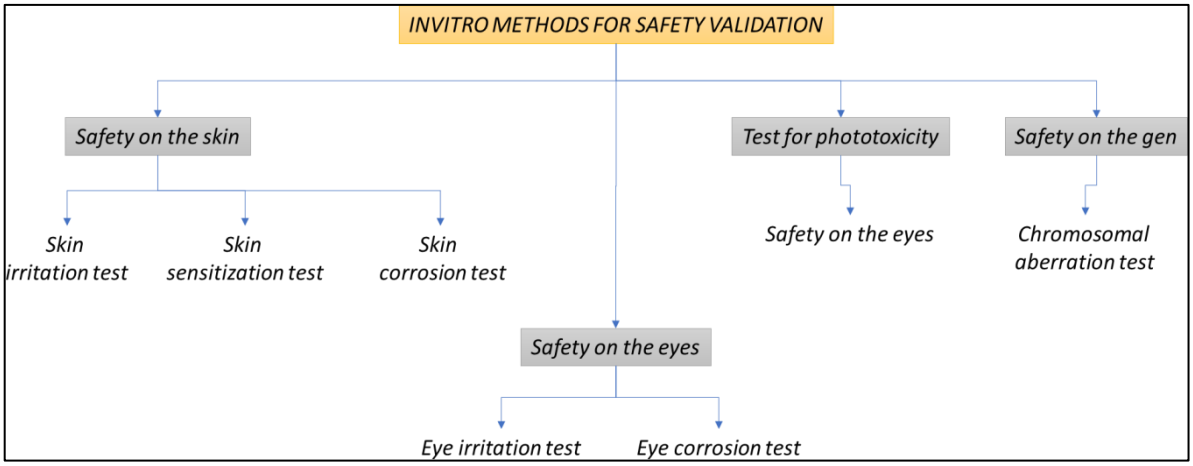

Figure-1 Methods for safety validation

In vitro Skin-irritation using Reconstructed Human Epidermis is test method

As per the United Nations (UN) Globally Harmonized System of Classification and Labeling of Chemicals (GHS), the reversible damage to the skin caused by exposure to a mixture or substance refers to skin irritation (https://unece.org/ghs-rev8-2019). The skin irritation potential of ingredients in the formulation is a critical factor in cosmetic safety assessment. In this test, the reconstructed RhE is used, which resembles the human epidermis and mimics its biochemical and physiological nature. The non- transformed human keratinocytes are used to create RhE, the final model is analogous to the In-vivo conditions as it consists of multiple layers of human epithelial cells[8]. Various concentrations of the test compound are directly applied to 3D-RhE model and cell viability is quantitatively assessed by enzymatic conversion of yellow dye MTT 3-(4,5-Dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide into a blue formazan salt[9-10]. **Figure-2** outlines the steps involved in, this assay.

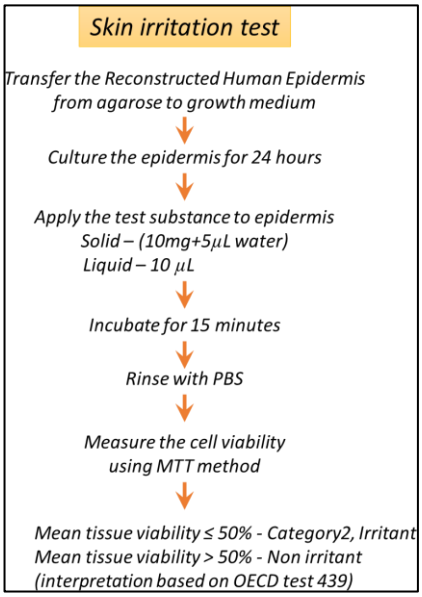

Figure-2 Skin Irritation Test

In- vitro 3T3 phototoxicity testing

Phototoxicity refers to the toxic elicited by photoreactive chemicals upon exposure to response environmental light. Cosmetic products may contain ingredients that are photo reactive and elicit an adverse response by the host. This is taken into account in an in vitro model system for assessing phototoxicity. As described in OECD test number – 432, 3T3 (Immortalized mouse fibroblast) cell lines are used for testing the phototoxic effects of the cosmetic ingredients. Cells are treated with test substances under two different conditions – one set of cells is not irradiated and the other set is irradiated. Further, neutral red dye is used to measure the viability of the cells. The IC_{50} values (the concentration of the test substance that causes 50% cell death) are calculated under these two conditions. The weak cationic neutral red dye is used to measure the viability as it can readily enter the membrane and accumulates in the lysosomes. Phototoxins induce cellular damage through reactive oxygen species and other mechanisms which alters the permeability of lysosomal membrane resulting decreased uptake and binding of Neutral red. The UV/vis spectrum of test chemical can be determined before testing *in-vitro*. Photo-cytotoxicity induced by test chemical is expressed quantitatively as reduction in the uptake of neutral red dye by cells in presence and absence of light with respect to solvent controls[11]. The chemical found to be photocytotoxic *in vitro* in this test can be phototoxic *in-vivo*. Some of the steps involved in this assay are outlined in **Figure-3.**

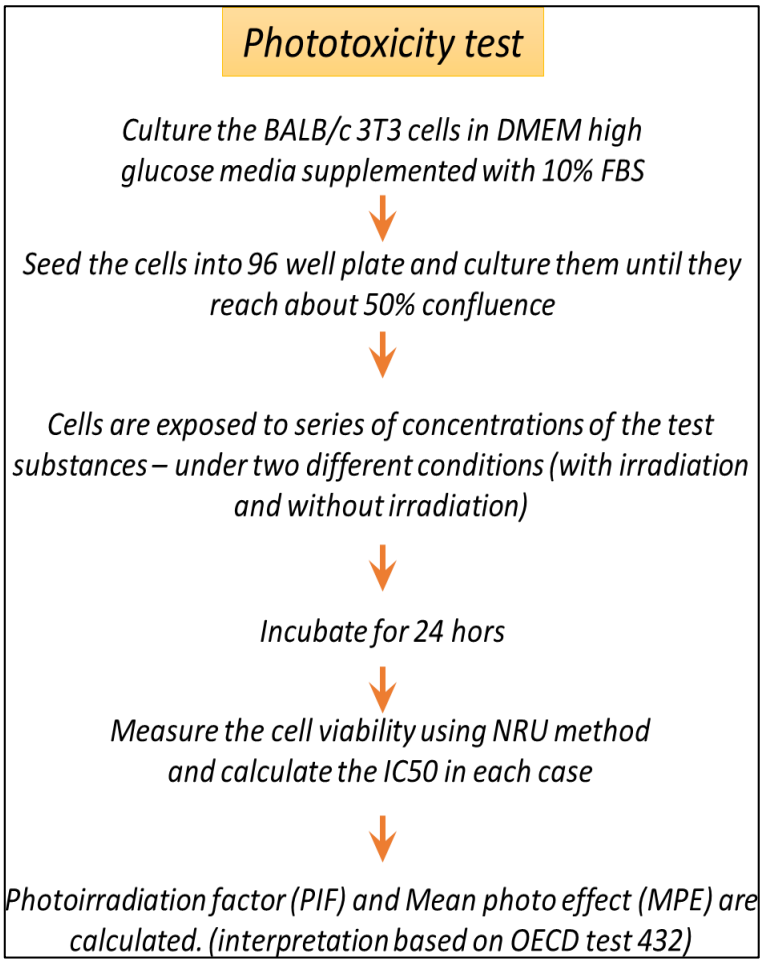

Figure-3 Phototoxicity test

In-vitro skin sensitization (Human Cell Line Activation Test, h-CLAT)

A skin sensitizer is any substance which induces allergic response following skin contact. This *in-vitro* test supports the discrimination between skin sensitizer and non-sensitizers. In h-CLAT, the sensitizer causes change in expression of cell surface markers resulting in activation of monocytes and dendritic cells h-CLAT method is useful to test the potential chemical with variety of functional groups, reaction mechanisms, and skin sensitization potency. As summarized in Figure-4, this test quantifies the change in expression of CD86 and CD54 on Human monocytic cell line (THP-1) following treatment with the test chemical. Flow cytometric analysis following cell staining with fluorochrome-tagged antibodies is used for measurement of changes in expression of cell surface markers.

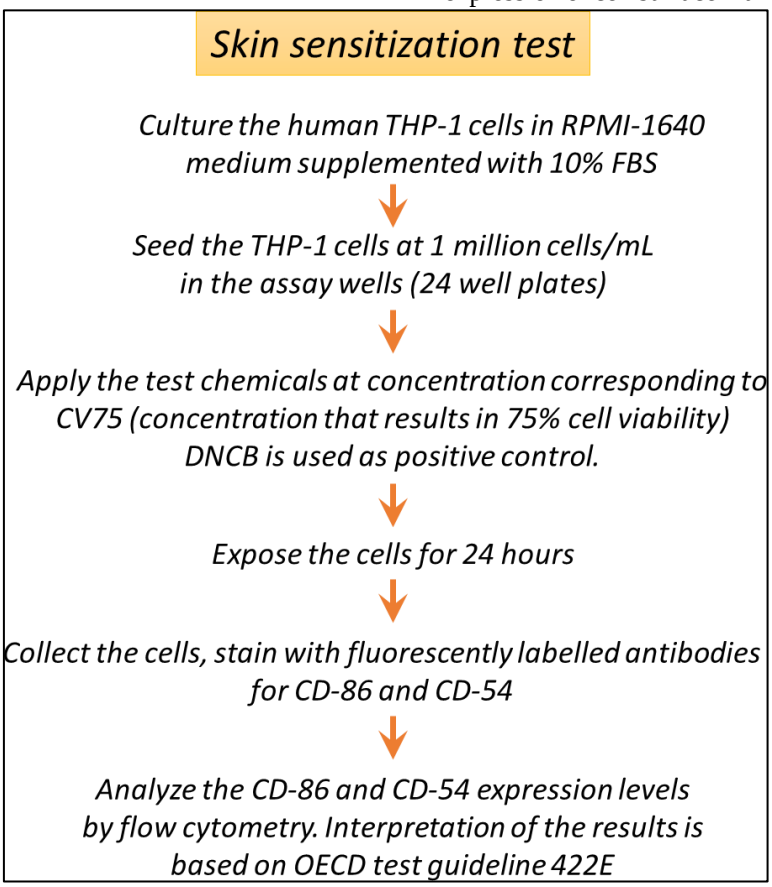

Figure-4 Skin Sensitization test

In -vitro Skin Corrosion Test

In this test, the 3D reconstructed human epidermis is used to identify potential skin corrosives following exposure time points of 3, 60, and 240 minutes. The human skin model assay works on the assumption that corrosive chemicals can penetrate the stratum corneum via diffusion or erosion and are cytotoxic to the underlying cell layers. The test material (solid or liquid) is applied topically and uniformly to a three-dimensional human skin model that includes at least a reconstructed epidermis with a functional stratum corneum. For each treatment (exposure time) and control, two tissue replicates are used. Corrosive materials are distinguished by their ability to reduce cell viability below defined threshold levels during specified exposure times. The cell viability is

assessed using MTT assay as end point. If cell viability is below 35% after 3 minutes of exposure time, the test chemical is corrosive as per sub- category 1A. The chemical will be considered as corrosive as per sub-category 1B and 1C if cell viability ≥ 35% after 3 min exposure AND < 35% after 60 min exposure OR ≥ 35% after 60 min exposure AND < 35% after 240 min exposure. In case cell viability remains ≥ 35% after minutes of exposure, the test chemical is considered non-corrosive[14].

In vitro eye irritation test (Short time exposure)

In this test, the sample is evaluated for its eye irritation potential using a confluent monolayer of Statens Serum institute Rabbit Cornea (SIRC) cells[12]. The cytotoxicity can be quantitively measured as the relative viability of SIRC cells following a five-minute exposure to test chemical. The reduction in cellular viability in SIRC cells following treatment with the test chemical is indicative of an adverse effect which may cause ocular damage. The cell viability is quantitatively assessed by enzymatic conversion of yellow dye MTT 3-(4, 5- Dimethylthiazol-2-yl) -2, 5-diphenyltetra-zolium bromide into a blue formazan salt. The hazardous potential of test chemical can be estimated by comparing the viability of treated cells with their respective solvent controls. The recommended exposed concentration of test chemical to the cells is 5%, 0.5%, and 0.05%. If the cellular viability at both 5% and 0.05% is less than or equal to 70% then the test chemical may have eye irritant properties and falls under category 1 chemical of the United Nations Globally Harmonized System of Classification and Labeling of Chemicals (UN GHS). The test chemical can be predicted as U N GHS No Category when the cellular viability is greater than 70% at both 5% and 0.05% concentration. Figure-5 outlines the steps involved in this assay.

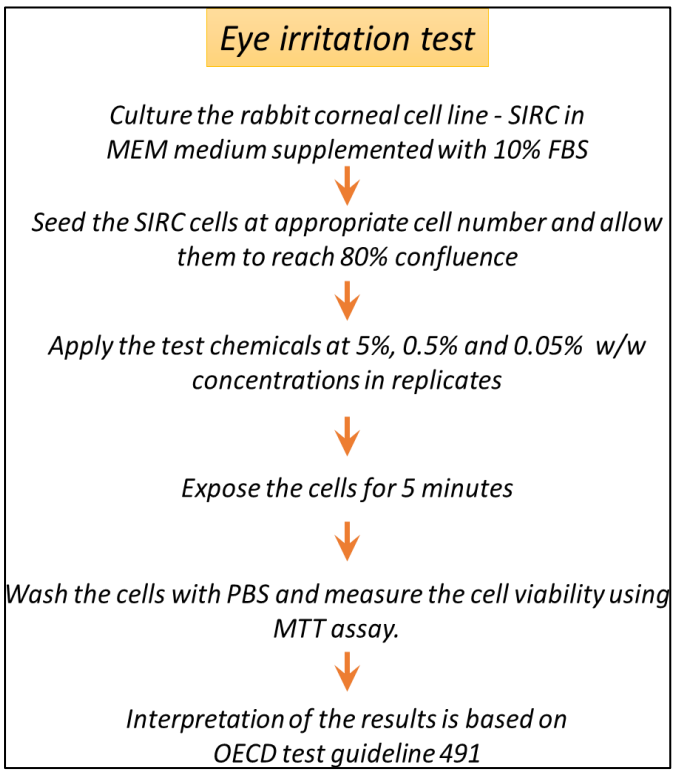

Figure-5 Eye irritation test

In vitro Fluoresce in Leakage Test Method

In this *in vitro* assay, the test chemical is assessed for its potential ocular corrosive nature and irritant effect[13]. This assay is performed on a confluent monolayer of Madin-Darby Canine Kidney (MDCK) cells grown on semi-permeable inserts. The MDCK cell line forms tight and desmosomal junction like the conjunctiva and corneal epithelia. The alteration in the permeability through these junctions, is the key event in chemical induced ocular damage. The leakage of fluoresce in through the junctions following test chemical treatment can be measured spectrofluorometrically. The amount of fluoresce in leakage is proportional to damage to the junctions and calculated with reference to fluorescence intensity of blank control and maximum leakage control. The concentration of test chemical (mg/ml) which produces 20% Fluoresce in leakage (FL_{20}) is considered as ocular corrosive and irritant. The chemical is ocular corrosive/severe irritant if the 20% Fluoresce in leakage (FL_{20}) is less than or equal to 100mg/ml.

In Vitro Mammalian Chromosomal Aberration Test

This test is to identify substances that cause structural chromosomal aberrations in cultured mammalian cells[15]. Structural aberrations are of two types: Chromosome and Chromatid. Primary cell cultures or the established cell lines of human or rodent origin can be used for this *in vitro* test. The stability of karyotype, capacity for cultured growth, and the frequency of chromosomal abnormalities are important factors while choosing the cells. Cell cultures are exposed to the test substance (liquid or solid) both with and without metabolic activation throughout roughly 1.5 typical cell cycle lengths. There should be at least three separate analyzable concentrations of the test substance used. It is recommended to utilize duplicate cultures for each concentration. Following exposure of cell cultures to the test drug, the cells are treated with a compound that stops the metaphase, collected, and stained. Cells that have been arrested in metaphase are examined under a microscope to see whether chromosomal aberration is present (Figure-6)

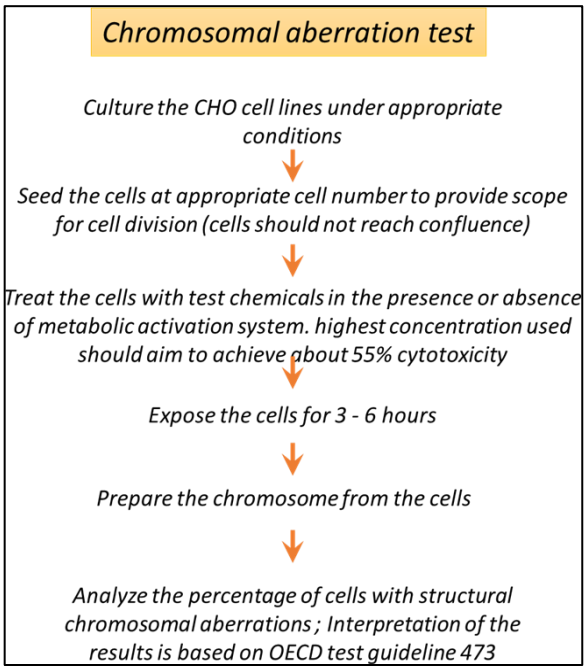

Figure-6 Chromosomal aberration te

Conclusion

Safety validation of the cosmetic products and ingredients is critical during the product development. As a matter of internal policy and owing to regulatory requirements, animal-based tests are not conducted in most of the industrial set ups for evaluating the safety of the cosmetic products. This necessitates extensive use of cell based systems. Several laboratories have been working on developing state of the art *in-vitro* methods that best mimics the physiological conditions. However, the non-animal based testing systems do have some limitations and some of the aspects of drug metabolism, absorption and toxico kinetics cannot be completely mimicked by these systems. In addition, the isolated cell based systems may lack physiological milieu which might be essential for capturing the effects of test substances. These aspects need to be carefully considered while setting up the safety assays for the cosmetic products. More than one assay system may be necessary before drawing the conclusion. With the advent of 3D skin technology, several drawbacks of the isolated cell based system can be overcome.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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Antibacterial activity of stem bark extract of traditionally used *Terminalia bellirica* (Gaerth.) Roxb.

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Abstract- Medicinal Plants have been practised for hundreds of centuries by tribes all over the world. From the earliest times until the end of nineteenth century plants are still the common source of medicinal treatment yet. Using natural, plant-derived medicines that are "healthier" than prescription drugs derived from synthesized products is something that appeals to consumers. In this study, the plant *Terminalia bellirica* was taken for study due to the mesmerizing medicinal properties of the plant. The antibacterial activities of acetone, ethyl acetate, benzene and methanol extracts of *Terminalia bellirica* stem bark were tested for four pathogenic bacterial strains by agar disc diffusion method. Among the various extract, acetone extracts showed good antibacterial activity and maximum zone of inhibition was obtained for *Staphylococus aureus* (zone size 8mm) followed by *Pseudomonas aurogenosa* (10mm). The results are given in **Table-1**. Methanol extract showed antibacterial activity against *E. coli* (zone size 8mm). On the other hand ethyl acetate and benzene extract was found to be totally unaffected against these bacterial strains.

Keywords: *Terminalia bellirica*, agar disc diffusion method, antibacterial activity, *Pseudomonas aurogenosa.*

Introduction

Infectious diseases caused by bacteria, fungi, viruses and parasites are still major threat to public health, despite the tremendous progress in human medicine. Their impact is particularly large in developing countries due to the relative unavailability of medicines and emergence of widespread drug resistance.[1] Research on new antimicrobial substances must therefore be continued and all possible strategies should be explored. Besides small molecules from medicinal chemistry, natural products are still major source of innovative therapeutic agents for various conditions, including infectious diseases.[2] *Terminalia bellirica* commonly known as belliric myrobalan belongs to the family combretaceae. It is routinely used as traditional medicine by tribal folk of

district Tehri Garhwal, Uttarakhand, India which lies in between $30^0$10' - $30^0$17' north latitude and $78^0$18' - $78^0$30' longitude. It is beneficial for the eyes, hair and cure jaundice, voice disorders, nasal problem, blood disorders, throat and breathing problems. Anthelmints seed of the fruit help to cure eyes problem. The fruit of *Terminalia bellirica* is one of the constituents of "Triphala", an Ayurvedic medicine.[3-4] The bark is useful in anemia and leucorrhoea Fruit of *Terminalia bellirica* causes fall in blood pressure of rats with its concentration of 70mg/kg body weight[5]. Chemical constituents, β-sitosterol, gallic acid, ethyl gallate, galloyl glucose, a new triterpene the belleric acid and chebulagic acid have been isolated from many species of *Terminalia* genus[6]. This prompted us to carry out the ethanobotanical study of the plant and antibacterial investigation of stem bark extract of traditionally used *Terminalia bellirica*.

Material and Methods

Clean and healthy bark of *Terminalia bellirica* were collected from District Tehri Garhwal Uttarakhand, India authenticated by Dr. J. K. Tiwari, Taxonomist, Botany department, HNB Garhwal University Campus Srinagar (Garhwal). Bacterial strains *Escherichia coli, Staphylococus aureus, Bacillus subtilis, Pseudomonas aerogenosa* were obtained from department of microbiology, Hill Campus Ranichauri and checked for purity by conventional biochemical method. Barks obtained were dried in tray drier for 7 days at 40-50^0 C and powdered.

Extraction

Powdered thus obtained was extracted with various solvent like, acetone, ethyl acetate, benzene and methanol using Soxhlet apparatus. The extract was concentration under reduced pressure to yield reddish waxy mass 80gm, 100gm, 90gm and 110gm and screened for their antibacterial efficacy against various pathogenic cultures by agar disc diffusion methods.

Antibacterial Activity Studies (Agar Disc Diffusion Methods)

The extract of stem bark of *Terminalia bellirica* was tested for antimicrobial activity using agar disc diffusion method on solid media[7-9]. Luria agar was used as basal medium for *Escherchia coli* and *Bacillus subtilis*; and nutrient agar was used as basal medium for *Pseudomonas aeroginosa*, *Staphylococcus aureaus*. 5 g of luria broth and 4 g of agar powder; 3.25 g of nutrient broth and 4 g of agar powder was weighed and 250 ml of water was added separately. The mixture was heated to dissolve the components. Luria agar and nutrient agar was sterilized in an autoclave[10]. Luria agar and nutrient agar was poured in the sterile Petri plates.

Mother cultures of each organism were set up 24 h before the assays in order to reach stationary phase of growth[11]. The tests were assessed by inoculating Petri dishes from the mother cultures which had been surface spread with 0.1 ml of each bacteria, with the aim of obtaining microorganism concentration of 10^5 colony forming units (CFU/ml)[12]. An aliquot of Dimethysulphoxide (DMSO) was added to the extract in order to obtain 5mg/ml concentration range[13]. Sterile dilutions of essential oil were deposited on the sterile Whatmann filter paper No.1 discs (5mm disc diameter), which were subsequently

placed in inoculated Petri plates. Therefore the Petri plates were than incubated at 37° C for 24 h. The antibacterial activity was determined by measuring the diameter of zone of inhibition surrounding bacterial growth[14].

Results and Discussion

The shade dried and powdered stem bark were exhaustively extracted with acetone, ethyl acetate, benzene and ethanol. A reddish waxy mass 80gm, 100gm, 90gm and 110gm obtained after removing the solvent from acetone, ethyl acetate, benzene and ethanol extract respectively. Under reduced pressure the above extracts were subjected for their antimicrobial activity. The stem bark extracts were found to be quite effective in inhibiting the growth of various bacterial strains as indicated by zone of inhibition. Among the various extracts, acetone extract showed good antibacterial activity and maximum zone of inhibition was obtained for *Staphylococus aureus* (zone size 8mm) followed by *Pseudomonas aurogenosa* (10mm), results are given in **(Table-1).** Ethanol extract showed antibacterial activity against *E. coli* (zone size 8mm). On the other hand ethyl acetate and benzene extract was found to be totally unaffected against these bacterial strains. Results of activity with zone of inhibition are shown in **Figure-1, 2, 3, 4.** Acetone extract is being subjected to column chromatography for isolation of the bioactive compounds for further studies.

Figure-1 Methanol extract against *Escherichia coli*

Figure-2 Acetone extract against *Pseudomonas aerugenosa*

Figure-3 Benzene & Ethyl Acetate extract totally in affected

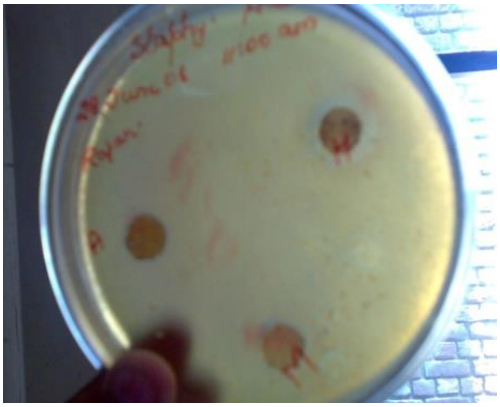

Figure- 4: Acetone extract against *Staphylococcus aureus*

Conclusion

It is concluded that the acetone extracts of stem bark of *Terminalia bellirica* are much more active against *Stephylococus aureus* and *Pseudomonas aerugenosa* (zone of inhibition 8mm, 10mm respectively), and methanol extract is active against *Escherichia coli* (zone of inhibition 8mm). Ethyl acetate & benzene extracts are totally unaffected for all the bacterial strains.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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Table-1 Antibacterial activity of Different extract of *Terminalia bellirica* stems bark

S. No.	Bacterial strain	Group	Zone of inhibition			
			Acetone Ext.	**Acetate Ext.**	**C_6H_6 Ext.**	**MeOH Ext.**
1.	*Bacillus subtilis*	Gram (+)	-ve	-ve	-ve	-ve
2.	*Staphylococcus aureus*	Gram (+)	8mm	-ve	-ve	-ve
3.	*Escherichia coli*	Gram (-)	-ve	-ve	-ve	8mm
4.	*Pseudomonas aerugenosa*	Gram (-)	10mm	-ve	-ve	-ve

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Extraction and dyeing of eco-friendly natural dye from Lodh bark on wool fabrics and optimization of procedure for dyeing using herbal mordants

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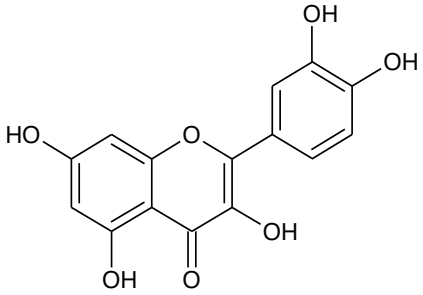
Abstract- The current study deals with the soaked wool fabrics were dyed with natural dyes extracted from the bark of *Symplocos racemosa*. The optimization was carried out for the different variables; concentration of dyematerial, time for extraction of dye, dyeing time, concentration of mordants methodsof mordanting. Out of the three methods of mordanting, the best shades for dye was obtained using pre-mordanting with lemon juice and vinegar andpost-mordanting with aqueous leaves extract of *Rhus parviflora* and aqueous leaves extract of *Erythrina suberosa* . The colour fastness tests indicated that all samples change in colour on exposure to light. The light coloured samples were affected more rapidly as compared to the dark ones. The Lodh bark dyed samples exhibited fairly excellent fastness to light and the results of washing fastness tests showed that dyed samples had good to excellent fastness to washing.

Keywords: *Symplocos racemosa* (Lodh), *Erythrina suberosa*, *Rhus parviflora*, Wool fabrics, Fastness, Herbal mordants, Natural dye.

Introduction

The art of dyeing with natural dyes[2,3,4] is very ancient[20]. There is very much evidence to indicate that dyeing had been practised in India[17], China and Persia, as far back as 2500 B. C. It is believed that some of the earliest traces of dyed textiles are found. Due to its eco-friendly environmental nature, it creates non-toxic environment and these plant pigments[5] have been used for dyeing textile, wool and fibers across the world by different human societies. In fact, natural dyes have been synonymous with traditional Indian textiles like the Kalamkari and Madhubani paintings and Pashmina shawls. In District Chamoli, Uttarkashi and Pithoragarh of Uttarakhand, traditional wool[11,12,15,16] and woolen products were still used for dyeing by the local villagers but there are certain problems with the use of natural dyes in textile dyeing[7,8,9,10] viz., colour yield,

complexibility of dying process, reproducibility results, limited shades, blending problems and inadequate fastness properties. Due to lack of availability of precise technical knowledge on the extracting and dyeing technique, it has not commercially succeeded like the synthetic dyes. Although indigenous knowledge system has been practiced over the years in the past, the use of natural dyes has diminished over generations due to lack of documentation. Also there is not much in format ion available on data bases of either dye-yielding plants or their products. Mordants either metallic salts or herbal mordants like extraction of leaves, roots, bark and flowers etc. may be used which produce an affinity between the fabric and the dye. Extract[6] of various plants, vinegar and lemon juices are the commonly used natural or herbal mordants. Colour fastness is the resistance of a material to change any of its colour characteristics or extent of transfer of its colorants to adjacent white materials in touch. The natural dyes[19] present in plants and animals are pigmentary molecules which impart colour to the materials. There are several plants that provide natural dyes[13,14,18] which are used in the textile industry. However, the common drawbacks of natural dyes are their non-reproducible and non-uniform shades, poor to moderate colour fastness and lack of scientific information on the chemistry of dyeing and standardized dyeing methods. Many reports are available on application of natural dyes on wool. *Symplocos racemosa* species is very rare to give natural dyes properties and this species give us good fastness grades with respect to grey scale. Isolation of natural dyes from these species under optimization[1,21] could not be done till now because recent data of the literature did not show these results of natural dyes properties in the past. The present study has been undertaken so as to revive the age-old are of dyeing with natural dyes. The present investigation deals with the aqueous extraction of natural dyes from the bark of *Symplocos racemosa* grow in almost all cold and dense parts of Garhwal Himalaya in Uttarakhand, India. The aim of present work has been carried out to prepare eco-friendly natural dyes the bark of *Symplocos racemosa* and then apply them on wool fabrics. The main aim of study is to get a cheap and easily available natural colourant for textile industry as well as to alter colour fastness of wool fabric sunder optimized condition and also to visualize the effect of metallic mordants have been undertaken. The structures of pre-isolated major colourant from this species have been mentioned below, **Figure-1**.



Quercetin

Figure-1 Chemical structures of pre-isolated dye bearing yellow colourant from the bark of *Symplocos racemosa*

Material and Methods

Collection of plant materials

Lodh bark were collected from the forests of District Chamoli of Uttarakhand, were dried in shade and crushed into fine powder form.

Wool- Wool in bulk was purchased from the tribal community of Gopeshwar, District Chamoli of Uttarakhand.

Mordants used as herbal mordants

The following non-toxic herbal mordants as aqueous extract of some plants materials like leaves of *Rhus parviflora* and flowers of *Erythrina suberosa,* vinegar and lemon juices were used as herbal mordants for the study.

Instruments used to analysis

The colour strength values of un-irradiated and irradiated dyed fabrics were investigated by CIE lab system using the Spectra flash (SF 660) at Chemistry Division, FRI, Dehradun, India. Colour fastness to washing of the dyed fabric samples was determined as per IS: 764 – 1984 methods using a Sasmira launder-O-meter following IS-3 wash fastness method. The wash fastness rating was assessed using grey scale as per ISO-05-A02 (loss of shade depth) and colour fastness to exposure to light was determined as per IS: 2454- 1984 method. The sample was exposed to UV light in a Shirley MBTF Microsal fade-O-meter (having 600 watt Philips mercury bulb tungsten filament lamp simulating day light) along with the eight blue cotton standards (BS1006: BOI: 1978). The fading of each sample was observed against the fading of blue wool standards (1-8). Colour fastness to perspiration assessed according to IS 971-1983 composite specimen was prepared by placing the test specimen between two adjacent pieces of wool fabric and stitched all among four sides as well as Grey scale was used for determining respective shades of colour of the dyed materials.

Scouring of wool

Wool yarn contain much grease and other impurities which affect the dye take-up by the fabrics, therefore it is desirable to give a thorough scouring treatment to the yarns, before being used for dyeing or mordanting. The scouring was done with a detergent solution prepared by mixing 0.5 of genteel with 100 ml of very hot water. The skeins were immersed in the prepared solution, after it had cooled to lukewarm temperature (40-50 ^{0}C). They were stirred with a wooden spoon for 30 minutes. The skeins were then removed, rinsed with lots of warm water again treated as above 3-4 and squeezing, till completely free of detergent. Care was taken not to scrub or mangle the skeins.

Preparation of skeins

The skeins were prepared so as to allow even preparation of the mordants and dye into each fiber. One gram of yarn was weighed and wrapped around a 10” cardboard piece. The resulting skein was removed and tied loosely at two places.

Optimization of different variables used in dyeing

The different variables like the concentration of dye material, time for extraction of the dye, dyeing time and the concentration of the mordants were optimized. The material-liquor ratio selected was 1:100. The optical density (O.D.) of the dye solution, before and after dyeing was recorded and the percent absorption was calculated by the following

formula:

$$\text{Percent absorption} = \frac{\text{O.D. before dyeing} - \text{O.D. after dyeing}}{\text{O.D. before dyeing}} \times 100$$

Concentration of dyeing material

The dye materials, in four different concentrations (2g, 4g, 6g, and 8g) were taken in the beakers containing 100 ml water. The extraction of the dye was done for 1 hour in boiling water and the solution was filtered. A sample of 2 ml was taken from each beaker and the optical density was recorded by diluting it 100 times. Four wool samples of 1g each were dyed in the solution for 1 hour at 100 ^{0}C and the optical density of the left over dye solution was recorded like as above. The results are given in the **Figure-2**.

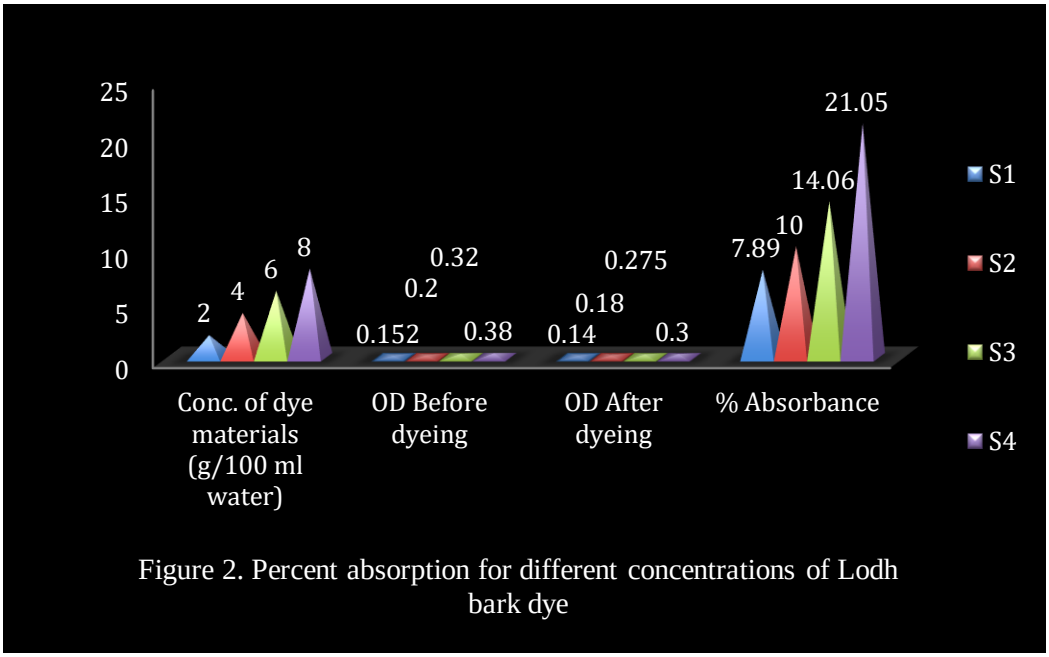


Figure 2. Percent absorption for different concentrations of Lodh bark dye

Time for extraction of the dye

Four beakers containing 100 ml of water were taken and the required amount of the dye material was put in each. The extraction of the dye was carried out at 100 ^{0}C for 15 minutes, 30 minutes, 45 minutes and 60 minutes. A sample of 2 ml was taken from each beaker and the optical density was measured. Four wool samples of 1g each were dyed in the dye bath and the optical density of the left over solution was recorded **Figure-3**.

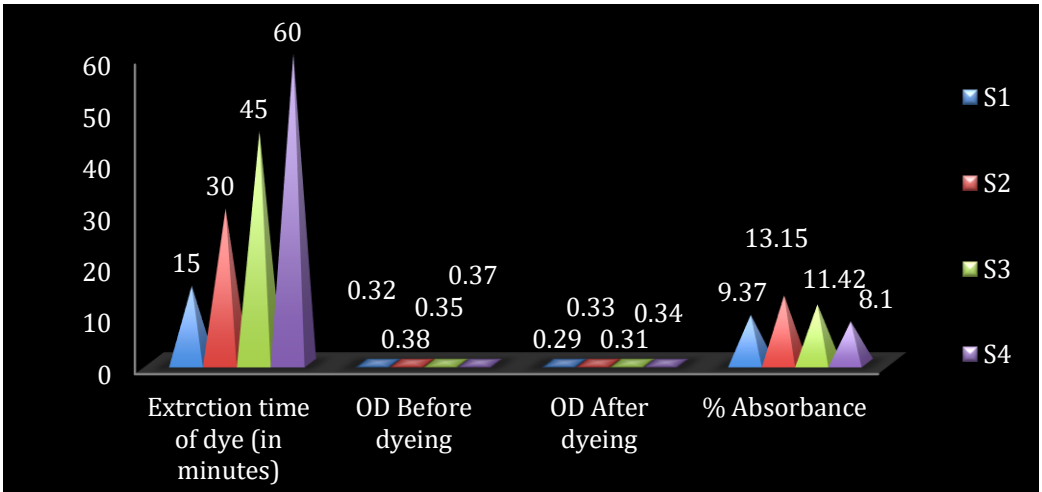


Figure 3. Percent absorption for different extraction time of Lodh bark dye

Dyeing time

The required amount of the dye material was put in four beakers containing 100 ml of water and the dye extraction was carried out at boiling temperature for the optimum time. The solution was filtered and a sample of 2 ml was taken from each beaker and the optical density was recorded. Wool samples of 1g each were put in the four beakers and dyeing was carried out at 100 ^{0}C for 15 minutes, 30 minutes, 45 minutes and 60 minutes. The optical density of each solution was recorded again **Figure-4**.

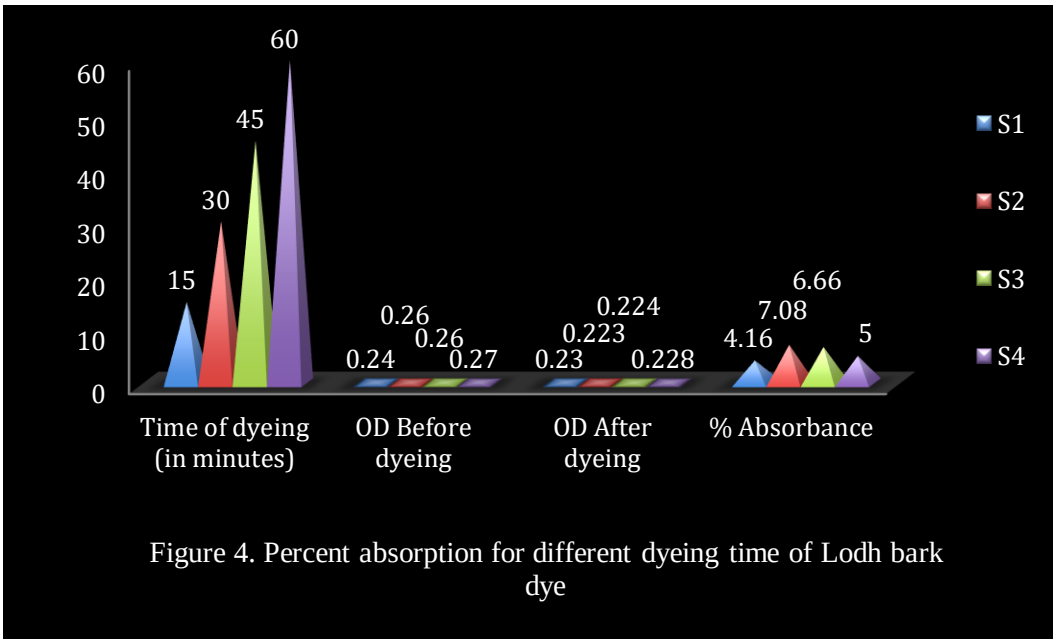


Figure 4. Percent absorption for different dyeing time of Lodh bark dye

Mordants

Organic dyes by their nature are fugitive and agents are therefore required to fix the dye molecules in the fabrics. These fixing agents are known as mordants. The mordant has a direct effect on the dye and governs the colour obtained, so that the same dye produces different colours when used with different mordants. The

following mordants were selected for the present study: leaves extract of *Rhus parviflora* and flowers extract of *Erythrina suberosa*, vinegar and lemon juice.

Concentration of mordants

The concentrations of all the mordants were taken 5 ml/100g of wool. The mordants in the above concentrations were put in beakers containing 100 ml of dye solution. After dissolving the mordant, 1g of wool was placed in each beaker and the dyeing and mordanting was carried out simultaneously at 100 ^{0}C for the optimum time. The percentages of ratings were calculated on the basis of the total marks obtained by each sample **Table-1**.

Table -1 Optimum concentration of different mordants for Lodh bark dye

S. No.	Mordants used	Conc. of Mordants (ml/100g of wool)	Percentage of rating
1.	Lemon Juice (LJ)	5	57.67
2.	Vinegar (V)	5	55.48
3.	Extract of *Rhus parviflora* (ERP)	5	58.45
4.	Extract of *Erythrina suberosa* (EES)	5	60.30

Preparation of dye liquor

Lodh bark dye: The required amount of dyes powder were put in beakers separately containing 100 ml of water and the extraction was carried out at 100 ^{0}C for one hour.

Preparation of blank samples

The weighed samples of wool yarns were soaked in water for 1-2 hours and added to the dye liquor prepared as above. The temperature of the bath was raised to boiling and the dyeing continued for 3/2 hours. The samples were allowed to cool in the bath, rinsed under tap water and dried in shade.

Methods of mordanting used

There are three methods of mordanting, out of which the optimum method was selected for each mordant, on the basis of visual evaluation, by a panel of judges. These are:

a) Pre-mordanting
b) Simultaneous mordanting and dyeing
c) Post-mordanting

Pre-mordanting

In this method, the required amount of each herbal mordants as 5 ml was poured in a beaker containing 150 ml of water. The pre-soaked and weighed samples were

placed in this beaker and the bath was gradually brought to boiling point. The yarns were stirred from time to time and the mordanting was continued for one hour. The samples were allowed to cool in the bath, removed, rinsed and dried in shade. The mordanted samples were dyed according to the methods prescribed for blank (3.7). The samples mordanted with non-toxic mordants as aqueous extract of leaves of *Rhus parviflora* and flowers of *Erythrina suberosa*, vinegar and lemon juice were dyed immediately after mordanting. Mordanting with aqueous extract of leaves of *Rhus parviflora* is very sensitive to light and hence special care

was taken to ensure that the dye bath was well covered.

Simultaneous mordanting and dyeing
5 ml of each herbal mordant were used and the solution was transferred to a beaker containing 150 ml of dye solution, and stirred for a few minutes. The pre-soaked sample was placed in it and the temperature was slowly raised to boiling point and the dyeing and mordanting was carried out for 1:30 hours. The samples were cooled in the bath, rinsed and dried in shade.

Post-mordanting
The pre-soaked samples were placed in beakers containing 150 ml of dye solution. The dyeing was carried out for 1:30 hours at boiling point. The samples were removed from the dye bath with a glass rod. 5 ml of each herbal mordant were transferred to the bath and mixed thoroughly with the dye solution. The samples were replaced in the dye bath and treated for one hour. They were allowed to cool in the dye bath, rinsed and dried in shade **Table-2**.

Table-2 Optimum method of mordanting with Lodh bark dye

S. No.	Mordants used	Mordanting methods	Percentage of rating
1.	Lemon Juice (LJ)	Pre-mordanting	63.26*
		Simultaneous mordanting	48.75
		Post mordanting	57.00
2.	Vinegar (V)	Pre-mordanting	55.56
		Simultaneous mordanting	47.00
		Post mordanting	62.88*
3.	Extract of *Rhus parviflora* (ERP)	Pre-mordanting	62.00*
		Simultaneous mordanting	51.25
		Post mordanting	50.45
4.	Extract of *Erythrina suberosa* (EES)	Pre-mordanting	45.20
		Simultaneous mordanting	55.15
		Post mordanting	68.50*

*** Best method of mordanting**

Preparation of final samples
The final samples for lodh bark dye was prepared by using the optimum concentration of dye material, optimum extraction time, optimum dyeing time, optimum concentration of herbal mordants and the optimum method of mordanting. These samples were used for preparing the sample sheets and for conducting the colour fastness tests.

Testing colour fastness of dyed samples

The final sample of lodh bark dye was subjected to colour fastness tests:

Test for colour fastness to light
To carry out this test, the dyed samples of 3 x 6 cm were mounted on a cardboard frame along with blue standards rated 1-8 respectively. The cardboard frame was

covered with a black sheet in such a way that all samples were half exposed and half covered. This frame was placed inside the fadeometer fitted with mercury bulb tungeston fluorescent lamp (MBTF) and faded as per the ISO recommendation. The standards and specimens were checked after every few hours till the fading was equivalent to grade 3 on the grey scale. The samples were compared with the blue standards and rated **Table-3.**

Test for colour fastness to washing

For this test the yarn was made into a sheet form of parallel length measuring 10 x 4 cm and placed between two pieces of undyed fabrics (of the same size), one of which was wool. All the three layers were sewn from all sides. The washing solution was prepared by dissolving 5 ml of detergent with 1 liter of water and the test was carried out according to the ISO recommended test No. 2, in the standard washing machine (launderometer). Each sample was treated for 45 minutes at 50 ± 2^0C, using soap solution in the ratio of 50:1. The samples were rinsed for 10 minutes in running water and dried in shade. The samples were assessed on the basis of change in colour of the samples as well as staining of the adjacent fabrics with the help of geometric grey scale **Table-3.**

Table-3 Colour fastness of samples dyed with Lodh bark dye

S. No.	Samples (Mordanted and Non-mordanted)	Rating for Washing fastness	Rating for staining	Rating for light fastness
			Wool samples	
1.	Control	4-5	4	4-5
2.	Lemon Juice (LJ)	5	4-5	4
3.	Vinegar (V)	4	4-5	4
4.	Extract of *Rhus parviflora* (ERP)	4-5	5	5
5.	Extract of *Erythrina suberosa* (EES)	4-5	4	5

Results and Discussion

The preliminary experiments showed that the bark of *Symplocos racemosa* yields a wide range of colours on wool fabrics.

Dyed and mordanted samples are revealing about dyed samples with lodh bark dye with different colour shades and so detailed experiments were conducted to standardize the methods of extraction and applications of the dyes on wool fabrics.

The results obtained by these tests are reported and discussed below.

Optimization of different variables

To optimize the dyeing procedure includes: Concentration of dye materials, Time for extraction of the dye, Dyeing time Concentration of mordants and Methods of mordanting.

Optimum concentration of dye materials

Four concentrations 2%, 4%, 6% and 8%, each of lodh bark dye was taken and the optical density (O.D.) of the dye liquor was recorded. The results obtained are reported in Figure-2 from which, it is evident that the percent absorption of lodh bark dye increased with increase in the concentration of dye up to 8% , on visual

inspection of wool samples dyed in the above concentrations, it was observed that best colour obtained when 8% dye solution was used (21.05). Hence this concentration was selected as optimum for further experiments. 8% dye solution is equivalent to 8gm of dye /100 ml of water/gm of wool.

Optimum time for extraction of dye

Crushed powder form of lodh bark was subjected to different periods of boiling and optical density of dye liquor for each period was recorded [Figure-3] which shows that maximum percent absorption (13.15), was obtained when lodh barks were boiled for 30 minutes, after which it steadily decreased as boiling was continued for 60 minutes (8.10). Wool samples gave best colour when the dye was extracted for 30 minutes and hence 30 minutes was selected as the optimum time for extracting lodh bark dye. Cavendish (1978) has reported that extraction time of natural dyes varies above 20 minutes. The observations of the present study are in accordance with those observations.

Optimum dyeing time

Wool samples were dyed for different periods of lodh bark dye solutions, optical densities for before and after dyeing were recorded. The results are given in Figure-4. It is clear from the Table-3 that initially percent absorption increased with the same time for dyeing wool samples, after some time it was decreased on continuation for the same time. Hence the best time for lodh bark dye was 30 minutes with maximum percent absorption as 7.08.

Optimum concentration of mordants

Wool samples with the same concentrations of mordants were used with lodh bark was visually evaluated by a panel of judges. There optimum concentrations were same but despite of that, samples revealed different percent of rating with different dyed and mordanted samples. There are four types of mordants were used as Lemon Juice (LJ), Vinegar (V), Extract of *Rhus parviflora* (ERP) and Extract of *Erythrina suberosa* (EES), among these four mordants Extract of *Erythrina suberosa* (EES) mordanted samples gave good percent of rating for lodh bark dye (60.30) and all above results are mentioned in the Table-1 respectively.

Optimum method for mordanting

The wool samples were mordanted with the three methods of mordanting: Pre-mordanting, Simultaneous mordanting and Post mordanting. Out of these the optimum method was selected for each mordant, on the basis of visual evaluation as in Table-2. It is clear from the Table-2, Lemon Juice (LJ) and extract of *Rhus parviflora* (ERP) were taken as mordants and applied with three mordanting methods like as above in which pre-mordanting method revealed maximum percent of rating, 63.26 and 62.00, which was the best mordanting method for lodh bark dye. Simultaneous mordanting with 48.75 and 51.25 and Post mordanting with 57.00 and 50.45 have low percent of rating than Pre-mordanting for the same. Secondly, Vinegar (V)and Extract of *Erythrina suberosa* (EES) were taken as mordants and applied with three mordanting methods in which maximum percent of rating was recorded in post mordanting, 62.88 and 68.50, rest two mordanting methods have less values than post-mordanting for the same.

Colour fastness of the dyed samples

Colour fastness to light

The rating of colour fastness to light was done with the help of geometric gray scale and the blue standards, by comparing the contrast between the exposed and unexposed position of the dyed samples. The results are reported in Table-3. It was observed that dyed samples with lodh bark dye as in Table-3, were mordanted with Lemon Juice (LJ) and Vinegar (V) showed fairly good fastness (4) while blank, Extract of *Rhus parviflora* (ERP) and Extract of *Erythrina suberosa* (EES) mordanted dyed samples showed excellent fastness to light (5).

Colour fastness to washing

The rating of washing fastness was done on the basis of change in colour of the samples as well as staining of adjacent fabrics as wool. On visual inspection with the help of gray scale, it was found that in case of lodh bark dye, the washing fastness was excellent for blank, Extract of *Rhus parviflora* (ERP), Lemon Juice (LJ) and Extract of *Erythrina suberosa* (EES) mordanted samples and while washing fastness was good for Vinegar (V). It was observed that for blank, there was almost slightly staining of adjacent fabrics for wool while there was almost negligible staining of adjacent fabrics as mentioned in Table-3.

Conclusion

The present study was undertaken to develop the dyeing process for dyeing wool fabrics with natural dyes obtained from bark of *S. racemosa* (lodh). The optimization was carried out for the different variables; concentration of dye material, time for extraction of dye, dyeing time, concentration of mordants methods of mordanting. It was concluded that 8% solutions of lodh bark dye gave the best results. Thus, it was selected as optimum concentration for dyeing 1g of wool samples. The optimum time required for maximum extraction of bark of *S. racemosa* (lodh) was found 30 minutes. The dyeing of wool samples for 45minutes yielded bright and beautiful shades for bark of *S. racemosa* (lodh) and there are concentrations of different mordants, which were same and it was found that 5 ml of extract of *Erythrina suberosa* (EES)/100 g for Tung leaf dye gave the best results. Thus, the above concentrations were selected as optimum for lodh bark dye. In the mode of mordant application, it was observed that when the method of mordanting was varied, there was a marked difference in the shades obtained. Out of the three methods of mordanting, the best shades for lodh bark dye was obtained using pre-mordanting with Lemon Juice (LJ) and extract of *Rhus parviflora* (ERP) and post-mordanting with Vinegar (V) and extract of *Erythrina suberosa* (EES). The colour fastness tests indicated that all samples change in colour on exposure to light. The light coloured samples were affected more rapidly as compared to the dark ones. The lodh bark dyed samples exhibited fairly good to good fastness to light and the results of washing fastness tests showed that lodh bark dyed samples had good to excellent fastness to washing. It may be concluded that these natural dyes can be used for dyeing wool on a small scale and may encourage rural women and youths to start a cottage industry based on the above methods. The beautiful shades can be used to satisfy the artistic and creative urge of

modern textile designers and can readily find a place in the colour schemes of today.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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Cyperus scariosus rhizome essential oil - physio-chemical characteristics and anxiolytic potential

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Abstract- Essential oils are important plant products that often have a complicated composition made up of the volatile plant components. Numerous biological actions are reportedly connected to essential oils, and a new class of aromaceuticals is being developed. The market for essential oils has increased since they are used to cure these types of ailments as a result of the emergence of various mental issues due to changing living standards, such as depression, anxiety, sleeplessness, and stress. Studies on animal models have shown that the mode of action of essential oils, which cause physiological changes in the brain, involves a number of neurotransmitter systems. Despite mounting proof that essential oils have quantifiable effects on animal behavior.

Therefore the current study is proposed to investigate the physical and chemical characteristics of the essential oil obtained from the rhizome of *Cyperus scariosus* and assess its anxiolytic effects on the central nervous system in rodent models.

Hydro distillation was used to extract the essential oil from the rhizomes of the plant. Total yield, physical properties like viscosity, specific gravity, refractive index, acidity, saponification, iodine no, etc were examined .Further to determine its impact on the central nervous system in a rodent model studies were performed at a dose level of 250 mg/kg body weight and compared to the benchmark medication caffeine @100 mg/kg body weight**.**

The essential oil has a sustainably stimulating impact on the central nervous system in a proportion of 88% compared to caffeine's 68%, according to CNS locomotors count and physicochemical features that were in good accordance with literature value.

Key words: *Cyperus scariosus,* anxiolytic, essential oil CNS.

Introduction

Essential oils are volatile complex compounds which are characterized by a strong odour and are formed naturally by aromatic plants as secondary metabolites. They are rich sources of biologically active compounds[1]. Essential oils are secreted in different parts of the plant. Essential oils

from a broad spectrum of plant species have shown antinociceptive, anti-inflammatory, antimicrobial, antiviral, antitumoral and antioxidant activities [2]. Some oil constituents can act as pro-oxidants (such as free fatty acids and hydroperoxides) or as antioxidants, including tocopherols, phenols, and possibly phospholipids together with other components. Moreover, recently there has been a profound interest in the antimicrobial characteristics of extracts from aromatic medicinal plants, specifically essential oils.

Essential oils are gaining remarkable interest for their multipurpose use as antioxidant, antifungal, and antiseptic agent; Study of various physicochemical characteristics explores the practical importance of herbal oils in daily life[3]. The quality of both fixed and essential oils is indirectly influenced by physical-chemical properties of the oil, such as colour, specific gravity, specific viscosity, refractive index, acid value, saponification value, ester value, carbonyl percentage, phenol and solubility tests, etc. The majority of an oil's commercial significance is determined by its physicochemical features, which provide a basis for assessing its suitability for consumption. The current study is proposed in order to investigate the physical chemical makeup of the essential oil extracted from *Cyperus scariosus*rhizome a very well known medicinal plant and assess its anxiolytic impact on the central nervous system in rodent models.Cypreus *scariosus* known by the common name "Nagarmotha" and Nagar Mustakais a small grass-like herb with angular softstem and underground rhizomatous tubers.Stolons are thin, measuring between 0.8 and 5.0 cm by 0.25 cm, and are covered in elliptic, acute, laxly striate, concolorous scales stem 40-90cm long, thin, and triquetrous at the apex, 1/8inch long1/24-1/16inch in diameter, variable, usually short (less than 1/3 inch. The rhizome of this plant contains anamber or light brown viscous essential oil .This premium essential oil-bearing plant is well-known around the world for its superior digestive and carminative properties. It is used to treate a variety of diseases including diarrhea, epilepsy, fever, gonorrhea, liver damage, syphilis and act as an important ingredient of several prescriptions used in indegenoius system of medicine.Oil reportedly kills intestinal worms effectively and also acts as a diuretic, astringent, anti-inflammatory, antibacterial, hypotensive, central nervous system stimulant, and antipyretic[4-10].

Material and Methods

Preparation of substrate-The rhizome of *Cyperus scariosus* was collected from a local mark of Dehradun authenticated by Systematic Botany Division, Forest Research Institute, Dehradun, Uttarakhand, India. The rhizomes were cut into small slice followed by oven drying at 60 °C - 80 °C for 48 hrs .The dried parts were then stored at room temperature and then used for the experiment.

Extraction of oil from leaves and rhizomes of *Cyperus scariosus*- In a Clevenger's apparatus, hydro-distillation (6-8 hrs) was applied to 300 g of Cyperus scariosus rhizomes. The resultant volatile fraction showed two separate layers: an upper aromatic oily layer and a lower colourless watery layer. These layers are transferred into separating funnel with

addition of diethyl-ether in it. The upper layer was collected and dried over anhydrous sodium sulphate.

Poured the solution into a small, dry, empty beaker, weighed it out, and then placed it over a water heater until all the ether has evaporated. The oil-filled beaker was again weighed to calculate the yield of oil from rhizome.

The oil was stored at low temperature (4 - 6°C) for further use[11].

Studies of characteristics of extracted oil[12]

Physicochemical characteristics provide a base line for aptness of oil. These characteristics of oil NM -1 studied were colour , Specific gravity, Specific Viscosity, Refractive index, optical rotation **,** acid number & saponification number[13-14]

(a) **Specific gravity**

1. A cleaned and dried empty pycnometer was taken and weighed.
2. The pycnometer was filled upto the mark with double distilled water and weighed again.
3. The weight of water was recorded. After removing the water the pycnometer was dried in the oven and filled upto the mark with the essential oil under experimentation.
4. The weight of pycnometer with oil was calculated using the following formula.

$$\textbf{Specific gravity} = \frac{wt.\ of\ the\ oil}{wt.\ of\ an\ equal\ volume\ of\ water}$$

(b) **Specific Viscosity**

1. An Ostwald viscometer was cleaned and dried. Ten ml of 1% solution (in acetone) of the oil under experimentation was filled in the bulb of the viscometer.
2. The solution was sucked upto upper mark and the viscometer left as much.
3. The time taken by the solution to percolate down from the upper mark of the viscometer to the lower mark was recorded.
4. The process was repeated by filling the viscometer with pure acetone. The relative viscosity of the oil was calculated as per following formula:

$$\eta\ \textbf{Rel} = \frac{\textbf{Flow time of 1\%solution of essential oil in solvent}}{\textbf{Flow time of pure solvent}}$$

The value of relative viscosity thus obtained, was converted to specific viscosity by the formula as under:

$$\eta\ sp = \eta\ rel^{-1}$$

where,

η sp = Specific viscosity

$\eta\ rel^{-1}$ = relative viscosity

(c) Refractive index

1. A type of refractometer was used to determine the refractive indices of the oils.

2.The double prisms of the apparatus were cleaned with alcohol and one drop of the oil was placed between them.

3. The prisms were closed by tightening the screw heads and the refractometer was allowed to stand for few minutes to equate the temperature of the oils and the apparatus.

4.The alidade of the refractometer was moved backward of forward to get a broader line which was a band of colour.

5.A sharp colourless line was obtained by rotating the screw heads of the compensator. Finally, the line was adjusted in such a way that it fell on the point of intersection of the cross-hairs.

6. The refractive index was head directly on the scales of the sector.

(d) Acid number- 1. One ml of the essential oil was dissolved in 15 ml of 95 % ethanol in a conical flask.

2.Three drops of 1% phenolpthaline were added to the contents of the flask and it was titrated against 0.1 N sodium hydroxide solution.
3. The first appearance of pink colouration that did not fade with in 10 second was considered as the end point.
4. Another set without oil was also run parallel to treatment and the difference in the amount of alkali used while titrating the treatment and the set without oil gave the amount of alkali consumed for determination of the acid number of the oil.
5. The acid number was calculated by the following formula :

$$\textbf{Acid number} = \frac{\textbf{Volume of 0.1 N Alkali consumed}}{\textbf{Weight of 1ml essential oil}} \textbf{ X 5.61}$$

(e) Saponification number- 1. One ml of the essential oil was taken into a 100 ml saponification flask. Ten ml of 0.5 N alcoholic sodium hydroxide solution was added to the flask and an air cooled glass condenser (1 meter in length and 1 cm in diameter) was attached to it.

2. The mixture was refluxed for an hour on a water bath and then allowed to cool down to room temperature.
3. The contents were the titrated against 0.5 N aquous hydrochloric acid using 3 drops of 1% phenolpathalin solution as the indicator.
4. Another set, without oil was also run parallel to the treatment set and the difference in the amount of acid consumed for the determination of saponification number of the oil, which was calculated by the following formula:

$$\textbf{Sap. No.} = \frac{\textbf{Volume of 0.5 N Acid consumed}}{\textbf{Weight of 1ml essential oil}} \textbf{ X 28.05}$$

(f) **Optical Rotation**- 1. Place the 100mm polarimeter tube containing the oil or liquid under the examination in the trough of the instrument between the polarizer and analyser.

2. Slowly turn the analyser until both halves of the field, viewed through the telescope, show equal intensities of illumination.
3. At the proper setting, a small rotation to the right or to the left will immediately caused a pronounced inequality in the intensities of illumination of the two halves of the field.
4. Determine the direction of rotation. If the analyser was turned counter-clockwise from the zero position to obtain the final reading, the rotation is laevo and if clockwise, it is dextro.
5. After the direction of rotation has been established, carefully readjust the analyser until equal illumination of two halves of the field is obtained. Adjust the eyepiece of the telescope to give clear, sharp lines between the two halves of the field.
6. Determine the rotation by means of protactor; read the degrees directly , and the minutes with the aid of either of two fixed verniers, the movable magnifying glasses will aid in obtaining greater accuracy.
7. A second reading should be taken; it should not differ by more than 5' from the previous reading.

Toxicity Study

Acute Toxicity Study[15]; For the evaluation of acute toxicity, five groups (n = 5) of male albino mice were employed. Animals from all groups were given a single dose of NH-1 (50, 100, 150, 250, 500, 1000, 1500, and 2000 Mg/Kg body weight) after fasting for the previous night. Animal behaviour was tracked during 72 hours following oil administration in a group of animals that had received an equivalent volume of P.B.S. Animals were watched for 14 days for any signs of toxicity and mortality was observed .

Antianxolytic Activity

The antianxolytic activity or locomotor activity (horizontal activity) can be easily measured using actophotometer which operates on photoelectric cells which are connected in a circuit with a counter. When a beam of light falling on the photocell is at on the animal a count is recorded. An actophotometer could have either a circular or square area in which the animal moves. Both rats and mice may be used for testing in this equipment.

Wistar rats of both sex (male and female) weighing 150-250 gms with a variation of ± 2.0 gm were taken for study. Test rats were kept in individual elastic ages with wire tops, prior to the use for screening. All the animals were fasted for at least 12 hours before use, allowing only access to water. The rats were divided into groups of five animals each. Each rat was weighed individually and was marked to distinguish one from another. The equipment was checked and was sure that all the photocell counter was recorded for each group for 10 mins. At the end of counting each group of rats were removed from the counting chamber. Drugs were given orally as per the following schedule and after 60 minutes they were retested for activity scores for 10 minutes. The dose for drug is 250 mg/kg body weight of rat while the dose for standard reference drug caffeine is 100mg/kg body weight. Animals were divided into 5 groups and received different drugs as Control group receiving Tween 80, Standard groups receiving caffiene and Test groups receivingNM-1 @ 250 mg/Kg body wt. Table 4

CNS motor activity was calculated as per the following formula: -

$$\% \text{ CNS activity} = \frac{\text{Initial no. of counts} - \text{Final no. of counts}}{\text{Initial no. of counts}} \times 100$$

Statistically Data

All data were expressed as mean SEM ± wherever applicable, the data were analysed statistically by student's t- test, using graph pad instant version 2.05a and one way ANOVA. The level of significance was $p < 0.05$ and n represents five per group.

Results

Various physicochemical properties viz. yield (%), specific gravity, specific viscosity, refractive index,optical rotation **,** acid number&saponification number are summarized in **Table-1**. Acute toxicity studies of Essential oil NM-1 was also carried out and further screened for their effect on Central Nervous System on rat model using actophotometer. Results are summarized in Table-2 and 3.

Table-1 Characteristics of NM-1

Parameter studied	Value NM-1
Yied	0.5%
Colour	Light brown
Specific gravity	0.9450
Specific viscosity	0.195
Refractive index	1.5106
Acid number	10.756
Saponification number	34.346

Table-2 Determination of LD50 of NM-1

Treatment	Dose (mg/kg PO)	No. of Animal	No. of Animal Dead	No. of Animal Survive	% dead animals
NM-1	50	5	0	5	0
	100	5	0	5	0
	150	5	0	5	0
	250	5	0	5	0
	500	5	0	5	0
	1000	5	0	5	0
	1500	5	0	5	0
	2000	5	0	5	0

Table-3 Antiaxyolytic (CNS) potential of NM-1

S.No.	Drugs	Dose	CNS Activity		% CNS Activity
			Before	After	
1.	Caffiene	100 mg/kg	37.6± 0.28	63.2±0.25	**68.08**
2	NM-1	250 mg/kg Body weight	40.6± 0.22	70.6±0.19	**88**

Discussion

Physicochemical properties analysis which indirectly influence the quality of essential oil revealed that, oils has comparable properties**.** Using an actophotometer, essential oil was further examined for acute toxicity tests and their impact on the Central Nervous System in a rat model. According to investigations on acute toxicity, sample NM-1 did not cause any toxic symptoms or mortality. at a dose of 50, 100, 150, 200, 500, 1000, 1500, or 2000 mg/kg body weight when given orally to mice. The aim of biological activity was to study effect of essential oil on the central nervous system, which was done in terms of locomotor activity of rats using actophotometer (activity cage).Most of the central nervous system acting drug influence the locomotor activities in human beings and animal the CNS depressant drug such as barbiturates and alcohol reduce motor activity while the stimulants such as caffeine and amphetamines increase the activity .in other words the locomotor activity on be in debt alertness of mental activity. Essential oils exhibit stimulation properties which lie in their structure closely in resemblance with actual hormones .[16-17]One of the important characteristics of these oils used in aromatherapy is their ability to penetrate the skin and reach the subcutaneous tissues. When breathed, essential oils are integrated into a biological signal of the receptor cells in the nose, which is how they work. Through the olfactory bulb, the signal is delivered to the limbic and hypothalamic regions of the brain. These signals trigger the production of neurotransmitters in the brain, including serotonin, endorphin, and others, that connect our nerve and other bodily systems to ensure the desired change and to give us a sense of relaxation. To have the desired effect on the body and mind, relaxing oil, euphoric oil, and stimulating oil, respectively, release serotonin, endorphin, and noradrenaline in adequate quantity thus gives stimulation signals. From activity data summarized in table3 . It is evident that in total NM-1 has exhibited CNS stimulant potential (88% @ 250 mg/ Kg body wtlocomotor count as compared to caffeine (68 % @ 100mg/ Kg body wt**)** locomotor count .

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Antimicrobial potentials of plant extracts against drug resistant bacteria

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Abstract-Antimicrobial resistance (AMR) is a serious and growing threat to human health. The development of new antibiotics is limited and slow. The tradition of synergy in herbal medicine is being used as a source of research ideas. The in vitro findings were that most of the research reported synergy both within plants and between plants and antibiotics. Whole plant extracts and combinations of compounds were shown to be more effective antimicrobials than isolated constituents. New sources of antimicrobial drugs need to be identified and improved strategy should be developed to combat multidrug resistance problem in pathogenic bacteria. Plant extract and phytochemicals demonstrating antimicrobial action needs to be exploited for their synergistic action between extracts and with antibiotics to exploit it in modern phytomedicine and combinational therapy. In the present study alcoholic extracts of medicinal plants were screened for their antimicrobial efficacy against drug resistant bacteria. The extracts of *Hemidesmus indicus,and Carum copticum,* showed promising action against one or more drug resistant bacteria with MIC ranged from 0.53 mg/ml to 7.80 mg/ml which has indicated their potential to be exploited in antimicrobial therapy and combination drug therapy after careful evaluation *in vivo* model.

Key words: Antimicrobial activity, MDR bacteria, MIC, Antibiotics, Antimicrobial resistance

Introduction

The World Health Organization (WHO) reports that there are internationally high levels of AMR in common bacteria alongside limited understanding and uncoordinated surveillance of AMR (WHO, 2014). There have been just two new classes of antibiotics developed in the last 40 years. The development pipeline is slow and although two new Cephalosporin combinations are expected to be licensed in Europe soon for use in humans, AMR will also emerge for these (O'Neil, 2015). Bacterial mechanisms for resistance are innate but the high correlation between antibiotic use and AMR is clear (ECDPC, 2015). Further research, development of collaborative working, novel approaches to prevent and treat infections and the exploration of possibilities for enhancing immunity (in relation to infection by bacteria) including using prebiotics and probiotics have been recommended (DOH and DEFRA, 2013). Research and approaches for improving human immunity and resilience have been lacking

(EUROCAM, 2014). WHO (2012) advises innovation and testing natural products to address AMR.

The use of herbal and other natural substances is part of the fabric of traditional medicine in different part of the world. Medicinal plants have been found good source of therapeutic and novel compounds.

Bacteria have evolved numerous defenses against antimicrobial agents and drug resistant pathogens are on the rise and such bacteria have become a global helath problem. Nearly twenty years ago over 90% *S. aureus* strains were reported β-lactamase positive. Strains of β-lacatam resistant *Staphylococcus aureus* including MRSA now pose a serious problem to hospitalized patients and their care providers (Liu, et al., 2000).. Similarly multidrug resistant problem is common in members of family Enterobacteriaceae specially *E.coli*, *Salmonella*, *Shigella* and several other humans and animal pathogen like *Haemophilus influenza*, *Campylo-bacter*, *Pseudomonas* aeruginosa, *Mycobacterium tuberculosis* both in developing and developed countries (Eldelstein *et al.,* 2001; Tonkic *et al.,* 2005;)India has one of the world's richest flora with about 120 families of plant comprising 1, 30,000 species. A large portion of the world population especially in the developing countries depends on the traditional-system of medicine for a variety of diseases. The world health organization (WHO) reported that 80% of the world's population rely chiefly on traditional medicines and major part of the traditional therapies involve the use of plant extracts or their active constituents (WHO 1993).

According to an estimate about 119 secondary plant metabolites are used globally as drugs. It has been estimated that 14-28% of higher plant species are used medicinally, that only 15% of all angiosperms have been investigated chemically and that 74% of pharmacologically active plant derived components were discovered after following upon ethanobotanical use of plants (Eloff, 1998). The plants are valuable in the three basic ways: (1) they are used as source of direct therapeutic agent. (2) As a source of new bioactive metabolites including antimicrobial, antihelminthic and antiprotozoan etc. (3) they serve as raw material base for elaboration of more complex semisynthetic chemical compounds.

According to a report published in the 'Journal of the American medical association', more than 630 million visit are made to alternative practitioners each year in the U.S. also more than 15 million adults take herbal remedies while taking other medication (Hoffman, 2004).

Concerted efforts have been made all over the world to explore the various biological and specific pharmaco-logical activities and their active compounds all over the world. However, targeted screening with improve strategy to evaluate the efficacy of various potential plants against problematic multi drug resistant bacteria is in the stage of infancy.

It is expected that plant extract showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. However very little information is available on such activity of plant extract (Lee *et al.*, 1998). In the recent years plants have been screened against multidrug resistant bacteria including *Staphylococcus aureus*, *Salmonella paratyphi*, *Escherichia coli*, *Shigella dysentriae* and *Candida albicans*. The selection of medicinal plant was based on their traditional uses in India and reported antimicrobial activity of many medicinal plants (Chopra *et al.,* 1992; Ahmad *et al.,* 1998; Mehmood *et al.*, 1999).

The recent development in the phytopharmacology is development of multicombinational drug against multidrug resistant bacteria. This has been possible due to interaction among plant extracts (Phytocompounds) and with other chemotherapeutic agents that may be synergistic or additive in their interaction. The development of these drugs has grown a new future in the area of phytopharmacology and medical practices.

At present multi drug therapy or combinational antibiotic therapy is in use. However its efficacy may be severely hindered against several MDR bacteria. Therefore, there is an increased request to develop novel drugs against multi drug resistant bacteria. One possible approach is to screen/unexplored Indian medicinal bioactive plant extracts for their potential to be used against multi drug resistant bacteria.

Considering the vast potential of Indian medicinal plants as an anti-infective agent, we have selected 15 plants on the basis of their traditional uses, ethano-pharmocological data and local availability. The present screening programme has been planned to identify most effective plants with broad spectrum activity against drug resistant microbial pathogens and to assess synergy with antibiotics *in vitro* in future studies.

Material and methods

Plants material

The authentic plant material was obtained from the Himalaya Wellness Company, Dehradun and identification of the plant samples was further confirmed by the plant taxonomist Dr. Maya Ram Uniyal, Former Jari Buti expert, Govt. of Uttarakhand.

Drug resistant and sensitive bacterial strains used in the screening programme

The Standard ATCC Culture strains were obtained from Hi-Media, Mumbai and clinical isolates were collected from Department of Microbiology, Himalaya Wellness Company. Multidrug resistant bacteria *Staphylococci* including methicillin resistant *Staphylococcus aureus* (MRSA), and Gram negative bacteria were also used in our laboratory.

Chemicals and Antibiotics

All the antibiotic discs were purchased from Hi-Media Lab Pvt. Ltd., Mumbai, India. The indicator dye p-iodonitro tetrazolium violet were purchased from Sigma Chemical Co., USA. MMS and Sodium azide were purchased from Sisco Reseach Laboratory, India. All the other media/chemicals used were of analytical grade.

Bacterial cultures

Bacterial isolates were obtained from different sources were subjected to antibiotic sensitivity by disc diffusion, method (Bauer et al., 1966).

Culture Media and Inoculum preparation

Nutrient broth/ Agar and Muller–Hinton broth/ agar (Hi-Media Pvt. Ltd., Mumbai, India) were used to grow the test bacteria at appropriate temperature 30-37 ^{0}C for 18hrs and then appropriately diluted in sterile 0.8% saline solution to obtain a cell suspension of 10^5 –10^6 CFU/ml.

Preparation of plant extracts and its fractionation

Plant extract was prepared as described earlier (Ahmad and Mehmood 1998) with a little modification. 800 gram of dry, plant powder was soaked in 2.5 liter of 70% ethanol, for 8–10 days and stirred after every 10 hr using a sterilized glass rod. At the end of extraction, it was passed through Whatman filter paper No.1 (Whatman Ltd., England). This alcoholic filtrate was concentrated under vacuum on rotary evaporator at 40 °C and then stored at 4 °C for further use. The crude extract was prepared by dissolving known amount of the dry extract in DMSO, to have a stock solution of 100 mg/ml concentration.

Antimicrobial assay

The agar well diffusion method (Perez et al. 1990) as adopted earlier (Ahmad and Mehmood 1998) was used. 0.1 ml of diluted inoculum (10^5 CFU/ml) of test organism was spread on Muller-Hinton agar plates. Wells of 8 mm diameter were punched into the agar medium and filled with 100µl of plant extract of 10mg/ml concentration and solvent blank (DMSO) separately. The plates were incubated at 37 °C, over night. The antibiotic (chloramphenicol) at 100µg/ml conc. was used in the test system as positive control. Zone of inhibition of bacterial growth around each well was measured in mm.

Minimum inhibitory concentration of plant extracts

Minimum inhibitory concentration of plant extracts against test bacterial strains was determined by tube broth dilution method, using specific dye (p-iodonitro tetrazolium violet) as an indicator of growth (Eloff 1998). 2 ml of the plant extract was mixed with 2 ml of Muller-Hinton broth (Hi-Media Ltd., Mumbai, India) and serially diluted into the next tube and so on. 2 ml of an actively growing culture of different test strains was added before incubating for over night, at 37 °C. After examining turbidity visually, 0.8 ml of 0.02 mg/ml indicator dye (p-iodonitro tetrazolium violet) was added to each tube and incubated at 37 °C. The tubes were examined for the colour development, after 30 min. Absence of growth was also confirmed by spreading 0.1 ml of broth from such test tube on normal nutrient agar plate.

Results and Discussion

Antimicrobial activity of plant extracts against drug resistance pathogenic bacteria

Multiple drug resistance in pathogenic bacteria has emerged as important problem in many countries of the world. There are now increasing case

reports documenting the development of clinical resistance to newer and broad spectrum antibacterial drugs like fluroquinolone (norfloxacin, cipro-floxacin, oflaxacin etc.) in many pathogenic bacteria. In the present study, clinical isolates of *S. aureus*,. and *E. coli*, were used. These microbial strains are found to be resistant to one or more antibiotics, showing the common occurrence of drug resistance **(Table-1)**.

Table-1 Antibiotics resistant pattern of test strains

Name of bacteria	**Strains code**	**Resistant pattern of used strains against antibiotics**
Staphylococcus aureus	SA-03	Cx, M, A. Pn, Cf, Do, Sm, Na
Staphylococcus aureus	SA-08	Cx, M, A, Pn, Cf, Sm,
Staphylococcus aureus	SA-11	Pn, Am, M, S, T, Do, Na, Cu,
Staphylococcus aureus	SA-21	Cx, M, A, Pn, Cf, Do, Sm,
Staphylococcus aureus	SA-22	Sensitive to all drugs
Staphylococcus aureus	SA-28	Pn, Am, Cx, Cf, M, Pc, Kt, T, S,
Staphylococcus aureus	SA-29	Cx, M, A, P,
E.coli	UP-2556	Pn, A, Cx, Do,
E.coli	EC-14	Pn, A, Cx, M, Ce, Cfx, Cep, Cu,
E.coli	EC-20	Pn, A, Cx, M, Ce, Cfx, Cu, Va, T, E,

Pn- Penicillin; A- Ampicillin; Cx- Cloxacillin; Ce- Cephotaxime; Cu- Cefuroxime; Cfx- Cefixime, Cefpodoxime; M- Methicillin; Va- Vancomycin; Nf- Nitrofurantoin; Nx- Norfloxacin; Nv- Novobiocin; Co- Co-trimoxazole; Na- Nalidixic acid; T- Tetracyline; C- Chloramphenicol; Do- Doxycycline and E- Erthromycin.

In the present study, 02 medicinal plants were selected on the basis of their traditional uses in treatment of different disease in India and worldwide. Only alcoholic extracts of plant material have been used as the alcohol was found suitable solvent for the extraction of antimicrobially active constituents from plants (Eloff,1998).

Antibacterial activity of crude extracts of the both medicinal plants against Gram positive bacteria (7 distinct isolates of *S. aureus*) and Gram-negative bacteria *E.coli* is presented in **Table-2 and Tabe-3**. Activity of ethanolic curde extracts against Gram positive bacteria showed broad spectrum antibacterial activity **(Table-2)**. On the other hand broad spectrum activity against Gram negative MDR bacteria was exhibited by *C. copticum*, followed by *Hemidesmus indicus* as evidenced from their activity against both test bacteria with fair size of zone of inhibition **(Table-3).** Most potential

plant extract was *Carum copticum* followed by *Hemidesmus indicus*. While activity of *Carum copticum, and Hemidesmus indicus* against MDR bacteria are probably reported for the Ist time.

Table -2 Antibacterial activity of plant extracts against Gram positive bacteria

Table- 3 Antibacterial activity of plant extracts against Gram negative bacteria

S. No	Scientific Name (Family)	Antimicrobial activity (Radius in mm)							
		SA-03	SA-08	SA-11	SA-21	SA-22	SA-28	SA-29	ATCC 6538*
1.	*Hemidesmus indicus*	21	20	23	22	23	21	19	28
2.	*Carum copticum*	26	24	22	25	26	20	18	30

S. No	Scientific Name (Family)	Antimicrobial activity (Zone in mm)	
		EC-14	EC-20
1	**Hemidesmus indicus**	22	20
2	*Carum copticum*	25	24

MIC values of *Carum. copticum* varied greatly from 0.53 mg/ml to 5.42 mg/ml against test bacteria. Similarly MIC ranged from 3.35 mg/ml to 7.80 mg/ml for *Hemidesmus indicus* **(Table-4)**. Variation in MIC values might be due to difference in cell well composition and intrinsic tolerance of the test isolates, nature and composition of phytoconstituents.

Our antimicrobial screening results also justify the traditional uses of these plants in ailments and localized skin infections caused by *S.aureus, E.coli,etc*.

Table -4 Activity profile of crude plant extracts in terms of Minimum inhibitory concentration (MIC)

S. No	Plant Extract	Yield in mg/100 gm of dry powder	Minimum inhibitory concentration against test microorganisms (mg/ml)								
			SA						EC		
			SA-03	SA-08	SA-11	SA-21	SA-28	SA-29	EC-M	EC-14	EC-20
1	**C. copticum**	6.38	4.6	5.42	4.42	0.53	1.48	4.17	4.14	2.04	2.05
2	*H.indicus*	4.36	6.7	7.80	5.42	3.35	4.34	6.89	6.34	4.56	5.76

Conclusion

This preliminary investigation indicated that potential plant extracts showing broad spectrum antimicrobial activity and synergy could be further tested to determine the efficacy *in vivo* against MDR bacteria. Active fractions of various plants may also be exploited in preparation of herbal formulation of improved efficacy and quality.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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Primary and secondary metabolites identification in *Hamelia patens* leaves

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Abstract-The aim of the present study was to investigate the presence of phytochemicals in leaves extracts (aqueous, methanol, ethanol) of *Hamelia patens*, the selected medicinal plant. Ethanolic and methanolic (organic) extract showed good number of compounds compared to aqueous extracts. Phyto-chemical analysis of organic extracts of *Hamelia patens* has shown the biological compounds like protein and amino acids, carbohydrates, cardiac glycosides, tannin, phenol, alkaloids, steroids, terpenoids, flavonoides and saponin. Quinones, phlobatannins, coumarins, emodins and fatty acids were absent in both the aqueous and organic extracts.

Key words: Phytochemical, *Hamelia patens*, Organic.

Introduction

The developing countries mostly depend on traditional plants focusing towards healthcare applications [Allameh, 2002]. Medicinal plants contain phytochemical compounds that are useful as medicine in controlling of various diseases and disorders. Plants always contain common source of medicaments, in traditional preparations or as pure active principles. Hence to identify plants or plant extracts that could be used to the drugs or that could replace some pharmaceutical preparations need to be purchased and imported [Farnsworth N R*et al*, 1985]. Ancient literature such as Rigveda, Yajurveda, Atharvaveda, Charak Samhita and Sushrut Samhita also describe the use of plants for the treatment of various health problems [Samy RP et al, 2008].

Hamelia paten is a large perennial shrub or small tree of the family Rubiaceae. Common names include firebush, humming bird bush, scarlet bush, and redhead [WELCH, 2003]. In Belize, this plant's name is Canaan and is also known as Guardian of the Forest. *Hamelia patens* is an ornamental plant grown almost worldwide in warm, moist areas. It is used to cure inflammation, rheumatism, headache and dysentery. [Surana and Wagh, 2015]. The plants are used in folk medicine for a range of ailments. The Firebush is used as herbal medicine to treat athlete's foot, skin lesions and insect bites and nervous shock [FENSTER, CHARLES B, 1991].

Material and methods

Collection of plant material

The leaves of *Hamelia patens* were collected from the Botanical Garden, Department of Botany, D.A.V (PG) College, Muzaffarnagar, UP, India.

Preparation of plant extracts

Preparation of sample and extraction were carried out as described by Saha *et al (2004)* with slight modifications. The fresh leaves samples were washed with distilled water and cut in to small pieces, shade-dried for 1 week followed by complete drying at 40 °C in oven. Then ground to from powder. 5 g of this dried sample from each variety was extracted separately with 50 ml of three different solvents: aqueous, ethanol and methanol for 24 h in a shaker at 50 rpm at temperature 30 °C. The extracts were filtered using Whatman filter paper and filtrates were used as an extract which was kept at 4 °C for the bioassay analyses.

Phytochemical screening (Qualitative method)

Standard methods to identify the phytochemical constituents as described by Sofowara (1993), Trease and Evans (1989), (Omoya and Akharaiyi, 2012), (Jyothiprabha and Venkatachalam, 2016). (Harborne and Williams, 2000).

Test for Carbohydrate- 2 drops of Molisch's reagent was added in 2ml of extract and shaken well. 2ml of conc. H_2So_4 was added on the sides of the test tube. A reddish violet colour ring appeared at the junction of two layers indicated the presence of observed.

Test for Steroids- 1 ml of extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by the sides of the test tube without disturbing the contents. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

Test for Proteins- 2ml of the extract were mixed with the 2ml of Burette reagent. A violet colour ring indicated the presence of peptide linkages of the molecule.

Test for Amino Acids- 2ml of the extract taken in tube and 2ml of in hydrin reagent was added into it and then kept this solution in hot water bath for 2-5 minutes. Appearance of purple colour indicated the presence of amino-acid in the sample.

Test for Alkaloids- The extract was mixed with the 6 drops of 1% HCl, development of precipitate indicated the presence of alkaloids in the sample.

Test for Flavonoides- 5ml of dilute ammonia solution were added to a portion of aqueous extract and then added conc. H_2SO_4. A yellow coloration was observed which confirmed the presence of flavonoides and it disappeared on standing.

Test for Terpenoids- 5 ml of extract was taken in a test tube and 2 ml of chloroform was added to it followed by the addition of 3 ml of concentrated sulphuric acid. Formation of reddish-brown layer at the junction of two solutions confirmed the presence of terpenoids.

Test for Cardiac Glycosides- 5ml of extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layer with 1ml of conc. H_2So_4. A brown ring of the interface indicated a deoxysugar characteristic of cardenolides. A violet ring might appear below the brown ring whereas the acetic acid layer, a greenish ring might form just gradually throughout thin layer.

Test for Tannins- 5ml of extract was added to few drops of 1% of lead acetate.

A yellow precipitate indicated the presence of tannins.

Test for Saponins- The extract mixed with 20ml of distilled water and agitated in a graduated cylinder for 15minutes. The formation of 1cm layer of foam indicated the presence of saponins.

Test for Phlobatannins- When an aqueous extract was boiled with 1%aqueous HCl, red precipitate was deposited which was taken as evidence for the presence of phlobatinins.

Test for Fatty Acids- 0.5ml of extract was mixed with 5ml of ether. These extracts were allow it for evaporation on filter paper and dried the filter paper. The appearance of transparency on filter paper indicates the presence of fatty acids.

Test for Anthocyanins- 2ml of aqueous extract is added to 2ml of 2NHCl and ammonia. The appearance of pink-red turns blue violet indicates the presence of anthocyanins.

Test for Leucoanthocyanins- 5ml of aqueous extract was added to 5ml of isoamyl alcohol. Upper layer appeared red in colour, this indicated the presence of leucoanthocyanins.

Test for Coumarins- 3ml of 10% NaOH was added to 2ml of aqueous extract formation of yellow colour indicated the presence of coumarins.

Test for Phenols- Take 2ml of extract to add 3ml of ethanol and a pinch of $FeCl_3$ to form greenish yellow colour this indicated the presence of phenols

Test for Quinones- 2ml of extract were mixed with 3ml of concentrated HCl to form green colour this indicates the presence of quinones.

Test for Emodins- 2ml of NH_40H and 3ml of benzene were added to the extract. Appearance of red colour indicated the presence of emodins in the test solution.

Results

The phytochemicals (primary and secondary metabolites) characteristics of *Hamelia patens leaves* extract were tested and summarized in the Table below.

Table- Phytochemicals (primary and secondary metabolites) screening of aqueous and organic extracts of *Hamelia patens* leaves.

S.No.	Phytochemicals	extracts		
		aqueous	methanol	ethanol
1	Tannin	+	+	+
2	Alkaloids	+	+	+
3	Cardiac glycosides	+	+	+
4	Quinones	-	-	-
5	Steroids	+	+	+
6	Terpenoids	+	+	+

7	Flavonoids	-	+	+
8	Fatty acids	-	-	-
9	Proteins	+	+	+
10	Amino-acids	+	+	+
11	Carbohydrates	+	+	+
12	Saponin	+	+	+
13	Phlobatannin	-	-	-
14	Phenols	-	+	+
15	Anthocynin	-	+	+
16	Leucoanthocyanins	-	+	+
17	Emodins	-	-	-
18	Coumarin	-	-	-

Proteins, sugar and chlorophyll are included in primary metabolites. They are essential for growth and survival of plants. Biosynthetically derived substances from primary metabolites like flavonoides, phenolic compounds, alkaloids, steroids, tannins, saponins, terpenoid are secondary metabolites. From the results, it was found that the various phytochemical constituents like protein and amino acids, carbohydrates, cardiac glycosides, tannin, phenol, alkaloids, steroids, terpenoids, flavonoides and saponin are present in organic (methanolic and ethanolic) extracts. This phytochemical screening is more prominent in ethanolic and methanolic (organic) extract as compared to aqueous extract as bioactive compound are organic in nature and soluble in organic solvent. Quinones, phlobatannins, coumarins, emodins and fatty acids are absent in both aqueous and organic extracts. Anthocynin and leucoanthocyanins both were only found in the organic extract. The bark of *Hamelia patens* contains significant amounts of tannins [FENSTER, CHARLES B. (1991)]. Flavonoid caused risk reduction mainly from cardiovascular diseases and cancer (Ballard & Marostica, 2019). The presence of classes of phytochemicals such as flavonoides, alkaloid, tannin showed cytotoxic effect (Chowdhury *et al.* 2017). The plants containing phenolic compounds could be useful as an antioxidant. cholesterol-lowering, as well as having cytotoxic qualities, anti-bacterial, anti-viral properties. These are credited to the presence of saponin (Bailly and Vergoten, 2020). The result suggests that the phytochemicals present in *Hamelia patens* extracts may show antimicrobial, anti-inflammatory and antioxidant properties.

Conclusions

Phytochemical screening played an important role in identifying various phytoconstituents present in plant extracts. It could be concluded that plants are a

great source of phytochemicals that could be utilized in curing various ailments. The study was only based on qualitative analysis and screening. Protein and amino acids, carbohydrates, cardiac glycosides, tannin, phenol, alkaloids, steroids, terpenoids, flavonoids and saponin were the phyto-constituents present abundantly in plants. The study provided an important basis for further investigation for the isolation and characterization of phyto-constituents from the selected plants for the development of drugs.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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Phytochemical screening and antimicrobial activity of *Aerva lanata* (Gorakh ganja)

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Abstract- *Aerva lanata* is a perennial shrub which belongs to the family Amaranthaceae and is found throughout tropical India as a weed in the fields and wasteland area. This plant is a good source of phytochemicals like terpenoids, flavonoides, alkaloids, phenolic compounds, glycosides, gums, tannins, terpenes, carbohydrates and amino acids. Other chemical constituents of *Aerva lanata* include ferulic acid, syringic acid, narcissin and feruloyl tyramine etc which are responsible for their antibacterial, antifungal, antioxidant, anti-asthmatic and anthelmintic activities. The result of phytochemical study shows that the alcoholic extract of *Aerva lanata* contains alkaloids, saponins, tannin, polyphenolic compound, amino acid, proteins, flavonoids, steroids, cardio glycosides, terpenoids and carbohydrate and it can be mentioned that the alcoholic extract of *Aerva lanata* contains higher number of phytochemicals as compared to all other extract of *Aerva lanata* studied in the present investigation. The MIC of the different extracts of *Aerva lanata* was found in the range of 25 µl to 45 µl. The different extracts of *Aerva lanata* shows antimicrobial activity against tested microorganism with inhibition zone ranging from 22 mm to 24 mm. The ethanol extract of *Aerva lanata* shows maximum zone of inhibition and is comparable or better than standard drug against all tested microorganisms.

Key words: *Aerva lanata*, Phyto-chemical and Amaranthaceous.

Introduction

Aerva lanata is a perennial shrub which belongs to the family Amaranthaceae and found throughout tropical India as a weed in fields and wasteland area. This plant is a good source of phytochemicals like terpenoids, flavonoids, alkaloids, phenolic compounds, glycosides, gums, tannins, terpenes, carbohydrates and amino acids. Phytochemicals are naturally occurring biologically active chemical substances present in plants. Proteins, chlorophyll and regular sugars are the primary metabolites whereas alkaloids, terpenoids, phytosterols, flavonoids, glycosides, tannins and phenolic compounds are secondary metabolites. Research shows that phytochemicals play an important role in protecting/curing of human's diseases. Phytochemical screening includes the extraction, analysis, and identification of the biologically active substances found in plants. It is most commonly used as Antiurolithiatic and diuretic for urinary disorders and for kidney stones. Research

studies show that betulin and quercetin of *Aerva lanata* having inhibitory property on enzyme activity which is responsible for kidney stone formation.

Other chemical constituents of Aervalanata include ferulic acid, syringic acid, narcissin and feruloyltyramine etc which are responsible for its antibacterial, antifungal, antioxidant, anti-asthmaticand anthelmintic activities. The other use of *Aerva lanata* are cytotoxic, anti-HIV, anti-tumor, anti-diabetic and anticancer.

Material and method

The aerial part of the plant is collected from the Himalaya wellness company, Faridabad. Mayers reagent, Hager's reagent, Molisch's reagent, Benedict's reagent, Fehling's reagent, Schiffs reagent sodium nitroprusside, NaOH, ferric Chloride, benzene, H_2SO4, chloroform, lead acetate, gelatin, HNO3, ferric chloride, Ninhydrin reagent, copper acetate, sodium bicarbonate, hydrochloric acid, litmus papers, Tollens reagent, iodine solution are analytical grade. Microbiological culture media and pathogen are purchased from Hi Media Laboratory Pvt. Ltd.

Preparation of extract

Take 10 g of dry powder of *Aerva lanata* in 500 ml round bottom flask (RBF) and add 200 ml of solvent (Ethanol, Water, Ethyl acetate, Chloroform and Hexane) for which extraction will do. Extract the material on water bath for 3 hours remove the flask and allowed to cool. Filter the supernatant through whatman filter paper no. 1 into clean beaker and kept on water bath to concentrate them. Concentrate till we get 50 ml of final concentrated extract through which we will do further analysis.

Phytochemical screening of extract

Standard methods are used for the screening of extract prepared with different solvent

Test for alkaloid

Mayer's Test- Take 5 mL of extract and adds 5 mL of 1% HCl, boiled on a water bath and then filtered. Take 2 mL of the filtrate add two drops of Mayer's reagent. Appearance of yellow precipitate indicates the presence of Alkaloids.

Wagner's Test- Take 5 mL of extract and add 5 mL of 1% HCl, boiled on a water bath and then filtered. Take 2 mL of the filtrate add two drops of Wagener's reagent. Formation of brown color precipitate indicates the presence of alkaloids.

Test for Carbohydrates

Molisch's Test- Take 2 mL extract and add 5mL of distilled water and filtered. To the 2 mL of filtrate add 2 drops of Molisch's reagent now add concentrated sulphuric acid along the walls of the test tube. Formation of violet ring indicates the presence of carbohydrates.

Benedict's Test- Take 2 mL extract and add 5mL of distilled water and filtered. To the 2 mL of filtrate add 2 drops of Benedict's reagent isadded and heated gently for two Minutes. Formation of red precipitate indicates the presence of carbohydrates.

Fehling's Test- Take 2 mL extract and add 5mL of distilled water and filtered. Take 2mL of filtrate and add 1mL of each Fehling solution A and B and boiled on a water bath for 2 min. Formation of brown

precipitate indicates the presence of carbohydrates.

Test for Glycosides

Legal's Test- 5 mL of extract is treated with 4mL of pyridine contained 2 mL of sodium nitroprusside solution. This is neutralized with 10% NaOH. Appearance of pink color shows the presence of glycosides.

Keller-kilani test- Keller-kilani test is carried out by mixing the 2 ml extract with 2 ml glacial acetic acid containing 1-2 drops of 2% FeCl3 solution. The mixture is then transferred into another test tube containing 2 mL concentrated H2SO4. Appearance of brown ring at the inter phase confirms the presence of cardiac glycosides.

Test for saponins

Foam Test- Take 5 mL of extract and add 20 mL of distilled water and shake vigorously in a 100 mL conical flask for 15min. Persistent foaming on shaking confirms the presence of saponins.

Test for Steroids

Salkowski's Test- Take 5mL of extract and add10 ml chloroform and filtered. To the filtrate add few drops of conc. H_2SO_4, shake and allowed standing. Appearance of golden yellow color indicates the presence of steroids.

Test for Phenolic compounds and Tannin

Ferric Chloride Test- Take 5 ml of extracts and adds 2-4 drops of 5% $FeCl_3$solution. Formation of deep green color indicates the presence of phenolic compounds.

Gelatin Test- Take 5 of extract and add 1% gelatin solution containing 10% NaCl. The formation of white precipitate indicates the presence of tannins.

Test for Flavonoides

Alkaline Reagent Test- Take 5mL of extract adds few drops of sodium hydroxide solution. Formation of an intense yellow color, which becomes colorless on addition of dilute acid indicate the presence of flavonoides.

Test for amino acid and proteins

Test for proteins- Take 5 ml extract add few drops of Conc. HNO_3. Formation of yellow color indicates the presence of proteins.

Ninhydrin Test- Take 5mL of extract add 15mL of distilled water. To this extract solution add 0.25% w/v Ninhydrin reagent and boiled for a few minutes. Appearance of blue color indicates the presence of amino acids.

Test for Terpenoids- Take 2 ml extract add dissolved in 2 ml chloroform and then evaporated to dryness. Now 2 ml of Concentrated H_2SO_4 is added to the resulting solid and heated for 2 minutes. The appearance of grayish color indicates the presence of terpenoids.

Anti-microbial activity

The following microorganisms were used for anti-microbial activity. *Bacillus subtilis, Streptomyces gresius, Staphylococcus aureus, Escherichia coli, Salmonella typhi*. All the microorganisms were maintained at 4°C on nutrient agar slants.

Determination of minimum inhibitory concentration (MIC) using micro-dilution method

The Minimum Inhibitory Concentrations (MICs) of different extracts were

determined based on the macro-dilution method (Berghe and Vlietinck, 1991) with some modifications as follows. The extracts were serially diluted (two-fold) in a series of test tubes using nutrient broth supplemented with 10% glucose and 0.05% phenol red (color indicator). These were later inoculated with 0.2ml suspension of the test organisms. The final concentrations were in the range 1000 to 10 µl/mL in the medium. Microbial growth was determined by observing for color change in the tube (red to yellow when there is growth). The lowest concentration that showed no change of color was considered as the MIC.

Anti-microbial activity

Cup plate method using Mueller-Hinton agar medium was employed to study the preliminary antibacterial activity of different extracts against different microbial strains. The Agar medium was purchased from HI media Laboratories Ltd., Mumbai, India. Preparation of nutrient broth, subculture, base layer medium, agar medium and peptone water was done as per the standard procedure. The cups each of 9mm diameter were made by scooping out medium with a sterilized cork borer in a petri dish which was streaked with the organisms. The extracts (100 µL) was added separately in the cups and petri dishes were subsequently incubated. Kenamycin (30 µg) were used as standard reference drugs. Zone of inhibition produced by different extracts were measured in mm.

Results and Discussions

Phytochemical screening of different extracts shows that all the phytochemicals are present in the alcoholic extract. Aqueous extract contains all the phytochemicals excluding steroids. Ethyl acetate extract contains all listed phytochemicals excluding carbohydrates and cardio glycosides. Chloroform extract contains only steroids and cardio glycosides. Hexane extract contain only steroids, cardio glycosides and carbohydrates as shown in the table given below.

Table Pytochemical Screening

Type of extract	Alkaloid	Saponins	Tannins & Polyphenolic compound	Amino acid & proteins	Flavonoids	Steroids	Cardio glycosides	Terpenoids	Carbohydrates
Ethyl acetate	+	+	+	+	+	+	-	+	-
Chloroform	-	-	-	-	-	+	+	-	-
Ethanol	+	+	+	+	+	+	+	+	+
Hexane	-	-	-	-	-	+	+	-	+
Water	+	+	+	+	+	-	+	+	+

Antimicrobial activity

Minimum inhibitory concentration of *Aerva lanata*

The MIC of the different extract of *Aerva lanata* are found in the range of 25µl to 45µl. The lowest MIC of ethyl acetate extract is found in the range of 30 to 35 µl against *Bacillus subtillis* and maximum MIC is in the range of 40-45 µl against *Streptomyces gresius*. The lowest MIC of chloroform extract is found in the range of 30 to 35 µl against *salmonella typhi* and *E. coli* whereas maximum MIC is in the range of 40-45 µl against *Stephylococcus aureus*. The lowest MIC of ethanol extract is found in the range of 25 to 30µl against *salmonella typhi* and *E. coli* whereas maximum MIC is in the range of 35-40µl against *Stephylococcus aureus*. The lowest MIC of Hexane extract is found in the range of 30 to 35 µl against *salmonella typhi*, *E. coli*, *Bacillus subtillus* and *Streptomyces gresius* whereas maximum MIC is in the range of 35-40 µl against *Stephylococcus aureus*. The lowest MIC of Water extract is found in the range of 35 to 40µl against *salmonella typhi*, *Bacillus subtillus* and *Streptomyces gresius* whereas maximum MIC is in the range of 35-40 µl against *Stephylococcus aureus* and *E. coli* as shown below in the (**Table-1)**.

Table-1 Minimum inhibitory concentration (MIC in µl)

Sr. No	Type of extract	Minimum inhibitory concentration (MIC in µl)				
		Bacillus subtillis	***Salmonella typhi***	***Staphylococcus aureus***	***Streptomyces gresius***	***Escherichia coli***
1	**Ethyl acetate**	30-35	35-40	35-40	40-45	35-40
2	**Chloroform**	35-40	30-35	40-45	35-40	30-35
3	**Ethanol**	35-40	25-30	35-40	30-35	25-30
4	**Hexane**	30-35	30-35	35-40	30-35	30-35
5	**Water**	35-40	35-40	40-45	35-40	40-45

The different extracts of *Aerva lanata* show antimicrobial activity against tested microorganism with inhibition zone ranging from 22 mm to 24 mm. the comparable or better results of different *Aerva lanata* extract with Kenamycin as standard drugs are as: ethyl acetate extract shows against *staphylococcus aureus* and *Streptomyces gresius*. The chloroform extract shows against *Streptomyces gresius*. The ethanol extract shows better result against most of all tested microorganisms. The hexane extract shows against *Streptomyces gresius*. The water extracts shows against *staphylococcus aureus* as shown in below **(Table-2).**

Table-2 The water extracts shows against *staphylococcus aureus*

Sr. No	Type of extract & Std Drug	Zone of inhibition (mm)				
		Bacillus subtillis	***Salmonella typhi***	***Staphylococcus aureus***	***Streptomyces gresius***	***Escherichia coli***
1	**Ethyl acetate**	18	19	20	18	17
2	**Chloroform**	14	15	15	17	14
3	**Ethanol**	22	25	23	21	22
4	**Hexane**	12	13	14	18	14
5	**Water**	13	15	19	14	15
6	**Kenamycin**	20	25	20	18	21

Conclusion

The result of phytochemical study shows that the alcoholic extract of *Aerva lanata* contains alkaloids, saponins, tannin, polyphenolic compound, amino acid, proteins, flavonoides, steroids, cardio glycosides, terpenoids and carbohydrate and we can say this type of extract of *Aerva lanata* contains more numbers of phytochemicals as compared to all other extract of *Aerva lanata* studied in the research under study. The ethanol extract of *Aerva lanata* shows maximum zone of inhibition and comparable or better than standard drug against all tested microorganism.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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Formulation and evaluation of Chocolate Lozenges for cessation of smoking

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Abstract- The use of *Avena sativa*, also known as oat straw, in herbal lozenges for smoking cessation shows promise. This herbal ingredient has a long history of medicinal use and has demonstrated potential in reducing cravings and withdrawal symptoms related to quitting smoking. The lozenges were prepared by using *Avena sativa* sugar and other excipients. The physicochemical properties of the lozenges were evaluated, including weight, thickness, hardness, friability, and dissolution rate. The lozenges were also evaluated for their sensory properties, including taste, texture, and overall acceptability. 100 grams of dark chocolate was melted properly and the drug was added to it and mixed well and then added in the mold for the perfect shape and allowed to cool

Keywords: Cessation of smoking, *Avena sativa*, Lozenges, Soft lozenges.

Introduction

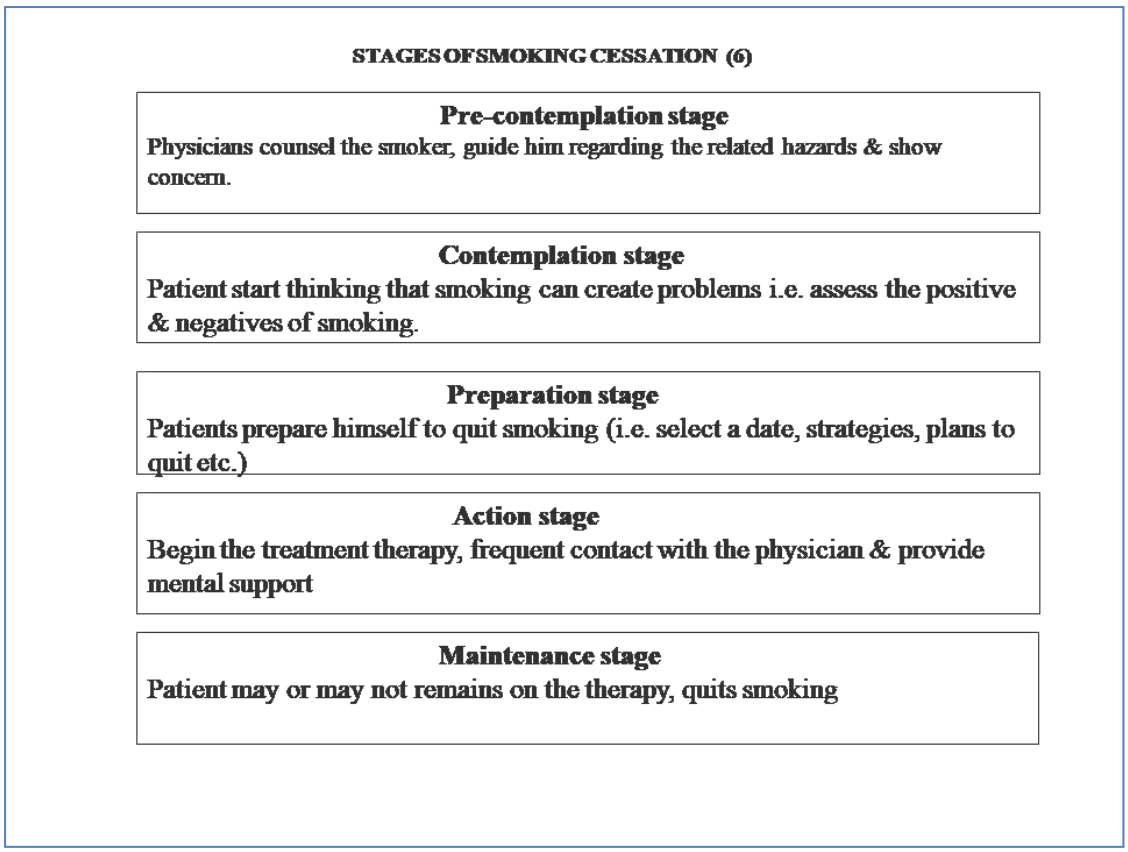
Smoking is the act of inhaling & exhaling the fumes of tobacco or any other burning plant material. Tobacco is the major cause of death and disability, leading to respiratory, cardiovascular, and cancer diseases. Smoking cessation is the most effective way to stop the advancement of COPD, as it is the most practical & effective way to do so[1,2,3] .

Nicotine is a nicotinic cholinergic agonist which at low doses binds to the nicotinic cholinergic receptors and emulates the effects of acetylcholine. At high doses, it produces a biphasic reaction characterized by stimulation followed by a depressant effect. It stimulates the release of dopamine, norepinephrine, acetylcholine, serotonin, pituitary hormones, and epinephrine. It also stimulates the cholinergic mechanisms which appear to impact upon the memory, alertness, and learning[4].

Smoking Cessation

Despite of the several warnings & graphical representation on the packaging itself that "Smoking is injurious to health", smokers found it difficult to quit smoking but it can be dealt with the following steps:

1. Drug Therapies
2. Behavioral Interventions
3. Other Interventions[5]



Accounting for the several health hazards of smoking, drug therapy has been increasingly relied upon to assist in smoking cessation[7].

Available Drug Therapy	Mechanism of Action
Nicotine replacement therapy	NRT works by reducing symptoms of nicotine withdrawal, thereby increasing the likelihood of smoking cessation
Anti-depressant therapy especially Bupropion	Bupropion is a weak dopamine and nor-epinephrine reuptake inhibitor, thereby maintaining central levels of dopamine through the process of cessation
Varenicline	Varenicline is a nicotinic acetylcholine receptor partial agonist, these could stimulate the release of sufficient dopamine to reduce craving and withdrawal while simultaneously acting as a partial antagonist by blocking the binding and consequent reinforcing effects of smoked nicotine.
Cytisine	Cytisine is a partial agonist selective for α4β2 nicotinic acetylcholine receptors, responsible for nicotine effects, and it prevents nicotine binding[5].

Recent advances have been made towards herbal therapies, complementary & alternative medicine, and traditional Chinese medicine for smoking cessation. Less-studied interventions such as acupuncture, aversive therapy, exercise, lobeline, mecamylamine, opioid agonists, anxiolytics, hypnosis, silver acetate are also being explored[8,9,10,11].

This study aims to review existing evidence related to efficacy and safety of herbal medicines on smoking cessation to make smoking cessation more accessible to all individuals. The herbal medicines with evidence to help in the smoking cessation situations are mixed herbal tea, aromatic black pepper extract, *Rauwolfia serpentina*, kava-kava, St. Johnswort, *Rhodiola rosea*, licorice, and *Rhodiola rosea*. Additionally, *lobelia inflata* can be used as an effective substitute during the initial withdrawal, and it can be used as a smoking blend or as a supplement. Smoking blends can also serve multiple functions, such as a reflective break from the day, control of oral urges, and continued social interaction. Gradually, the amount of lobelia in the blend can be decreased[12].

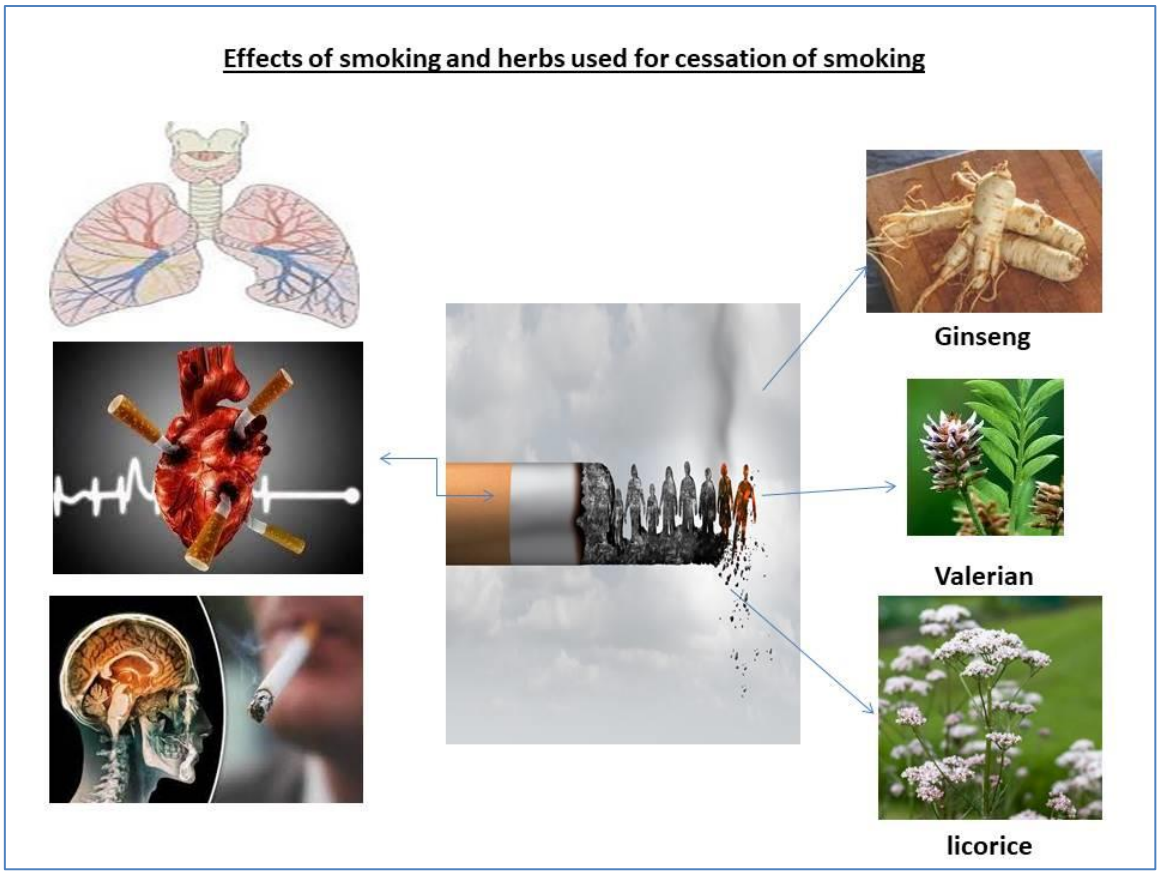


Figure-1 Smoking effects and role of herbs.

Material and methods

Table-1 Formulation of Lozenges[13]

S. No	Ingredient	Quantity Used
1.	Drug	10 G
2.	Chocolate	100 G

Preparation of soft lozenges

Chocolate weighing around 100 gm was melted using a water bath and then approximately 10 gm of crude extract was added to it. The mixture was stirred and thoroughly blended before being poured into molds. Subsequently, it was left to cool down and solidify at room temperature[14,15,16].

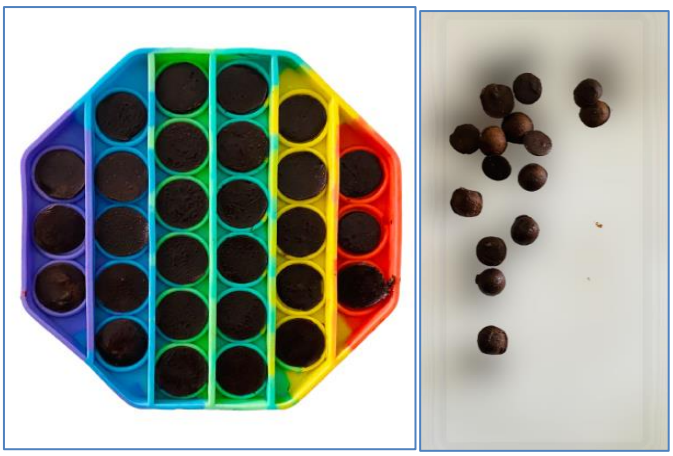


Figure-2 Lozenges in a mold and chocolate lozenges.

Quality control of lozenges[17]

1. Hardness test- The firmness of lozenges is measured using either a Pfizer or Monsanto hardness tester. The ability of lozenges to withstand shipping or breakage during storage, transportation, and handling prior to use is dependent on their hardness.

2. Diameter and thickness: The tool utilized to measure the diameter and thickness of lozenges is a Vernier caliper.

3. Weight variation: In this study, 20 lozenges were chosen randomly, and each one was weighed individually with an electronic balance. The mean weight and standard deviation were calculated for the 26 tablets, or the initial weight was compared to the computed average weight.

4. Disintegration test: The USP Disintegration apparatus is utilized to determine the disintegration time of lozenges, which is recorded in either pH 6.8 phosphate buffer or artificial saliva at a temperature of 37ºC. In an in-vitro drug dissolution study, the rate of drug absorption is determined by the rate of drug dissolution of the lozenges. The efficacy of the lozenges is directly related

to their rate of dissolution and bioavailability. This study is carried out using the USP II Dissolution type apparatus, which is a paddle-type instrument. The dissolution study is conducted in 900 ml of buffer pH 6.4 or artificial saliva by the USP II paddle method at 100 rpm. Samples are taken at 5-minute intervals and immediately replaced with an equal volume of fresh buffer or artificial saliva. The samples are then analyzed spectrophotometrically, and the temperature is maintained at 37^0C ± 2^0C during the dissolution studies.

Results and Discussion

Table-2 Evaluation

S.No	Tests Performed	Result
1.	Shape	Semi-circle
2.	Color	Brown
3.	Taste	Sweet
4.	Flavor	Chocolate
5.	Hardness	5 kg/cm^2
6.	Diameter	Avg. 14 mm
7.	Thickness	Avg. 0.66 mm
8.	Average weight	1.16 g (0.5 g weight variation)
9.	Disintegration time at 30 °C	7 minutes 15 seconds

Conclusion

The preparation of herbal lozenges using *Avena sativa* as a potential aid for smoking cessation shows promise. *Avena sativa*, commonly known as oat straw, has been used for its medicinal properties for centuries and has been found to have potential benefits in reducing cravings and withdrawal symptoms associated with smoking cessation.

The formulation of *Avena sativa* based lozenges requires careful consideration of the quantity and quality of ingredients as well as the manufacturing process. Factors such as the optimal temperature and duration of heating, mixing techniques, and the selection of excipients can influence the efficacy and safety of the final product.

Although more research is needed to confirm the effectiveness of *Avena sativa* lozenges in smoking cessation, initial studies have shown promising results. Overall, the development of herbal lozenges using *Avena sativa* represents a potential alternative or complementary approach to traditional smoking cessation methods and warrants further investigation.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research.

There is no conflict of interest between authors and producers of the product.

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About flowers on the cover page

Sesamum indicum (Sesame)

Scientific name: *Sesamum indicum*
Higher classification: Pedaliaceae
Rank: Genus
Order: Lamiales
Kingdom: Plantae

Sesamum is a genus of about 20 species in the flowering plant, family Pedaliaceae. The plants are annual or perennial herbs with edible seeds. The best-known member of the genus is sesame, Sesamum indicum, the source of sesame seeds.

The sesame plant, *Sesamum spp.*, is spread throughout the tropical and subtropical areas in Asia, Africa, and South America. Sesame seeds are rich in oil, protein, mineral ash, crude fiber, oxalates and soluble carbohydrates and phytate. Sesame oil is used in various edible applications, as a solvent for intramuscular injections and in the production of drugs, perfumes, cosmetics, creams, lubricants, insecticides, and fungicides. Sesame oil is unique because it contains the furo fuanlignan sesamin and its analog sesamolin,

which together can comprise 0.5%–1.5% to the oil. These ligans can affect the desaturation and β-oxidation of fatty acids as well as inhibit cholesterol biosynthesis and tocopherol metabolism and excretion. These effects may exert beneficial health effects.

Terminalia bellerica (Baheda)

Scientific name: *Terminalia bellirica*
Family: Combretaceae
Higher classification: Tropical almond
Rank: Species
Kingdom: Plantae
Order: Myrtales

Terminalia bellirica, known as baheda, bahera, behada, beleric or bastard myrobalan, Persian بلیله, Sanskrit: Vibhītaka □□□□□□, Aksha □□□□ is a large deciduous tree in the Combretaceae family. It is common on the plains and lower hills in South and Southeast Asia, where it is also grown as an avenue tree

The fruits have laxative, astringent, anthelmintic and antipyretic properties and are used in Ayurveda against various disorders like hepatitis, bronchitis, asthma, dyspepsia, piles, diarrhea, coughs, eye diseases.

Cyperus scariosus (Nutgrass)

Scientific name: *Cyperus scariosus*
Family: Cyperaceae
Order: Poales
Kingdom: Plantae

Cyperus is a large genus of about 700 species of sedges, distributed throughout all continents in both tropical and temperate regions.

This astringent plant, sharp in taste with cooling properties, induces perspiration, urination (and constipation). Root: Tubers used for phlegm, bile, fever and bowel problems. Their use protects against loss of appetite, thirst, burning sensation, and asthma. The paste is also used for nausea, gastric ailments, sour stomach, swollen limbs, itching, leprosy, herpes, and scabies. Tuber powder is used to relieve the swelling caused by scorpion venom. Drinking the milk made by stewing tubers in milk and water until only milk is left provides a cure for dysenteric stomachaches with discharge of mucus or diarrhea with of bits of blood.

Cichorium intybus (Chicory)

Scientific name: *Cichorium intybus*
Family: Asteraceae
Subfamily: Cichorioideae
Higher classification: Cichoriinae
Rank: Genus
Order: Asterales

Cichorium is a genus of plants in the tribe Cichorieae within the family Asteraceae. The genus includes two cultivated species commonly known as chicory or endive plus several wild species. Common chicory is a bushy perennial herb with blue or lavender flowers.

Cichorium intybus, commonly known as chicory, is well known as a coffee substitute but is also widely used medicinally to treat various ailments ranging from wounds to diabetes and is effective for hepato-protective use in ayurvedic formulation for liver ailments.

Review

2023 The International Year of Millets
(India sponsored resolution unanimously adopted by 75th United Nations General Assembly session)

Miracle Grains of Future

The plant, Millet belonging to the family Poaceae was thought to be amongst the most suitable plants to be domesticated and consumed as traditional crop by farmers in the rain scanty areas of Asia and Africa for more than 4500 years, India being on the top as its producer followed by Niger, Nigeria and China. During the middle period, this plant was used to provide major grains in Europe too whereas today it is used mainly to produce hay in Western Europe and U.S.A.

The plants of this cereal are annuals 25 to 125 cms in height. They are rich in carbohydrates with protein 7% to 10% and fat 2 to 5

and are least exploited food consumed in the form of rice, porridges and round flat breads. The grains of these cereals of late have been incorporated in other foods and made traditional.

There are about nine types of millet in India viz; Pearl, Sorghum, Barnyard, Browntop, Little, Proso, Finger, Foxtail and Kodo millets.

Types of millet in India

Millet is one of the neutraceuticals which provides several health benefits, prevention and treatment of diseases. Grains of millet are rich in nutrients and phenolic compounds. The diverse percentage of nutrients and phenolic compounds present in the pearl and finger millet indicate its different varieties viz; of phenolic acids, tannins and flavonoids which are useful for health. Pearl millets have shown less unique abundance and diverse phenolic properties as compared to those of finger millets. Research work has indicated that millet phenolic properties are highly antioxidant. Phytochemicals present in the millet grains show positive effect on human health by reducing cholesterol and phytates in the body as well as defend the cereal grains against virus, bacteria and pathogens.

Dr. I. P. Saxena
Editor (UJPAH)

Forthcoming Events

1. **International Conference on Phytochemistry and Natural Products (ICPNP) - Tokyo, Japan**
Website URL: https://waset.org/phytochemistry-and-natural-products-conference-in-july-2023-in-tokyo
Date: Jul 22, 2023
2. **International Conference on Cancer Pharmacology and Applications (ICCPA) - Montreal, Canada**
Website URL: https://waset.org/cancer-pharmacology-and-applications-conference-in-august-2023-in-montreal
Date: Aug 05, 2023
3. **International Conference on Phytochemistry and Natural Products (ICPNP) - Tokyo, Japan**
Website URL: https://waset.org/phytochemistry-and-natural-products-conference-in-august-2023-in-tokyo
Date: Aug 16, 2023
4. **International Conference on Traditional Medicine, Pharmacognosy and Phytochemistry (ICTMPP) - Prague, Czechia**
Website URL: https://waset.org/traditional-medicine-pharmacognosy-and-phytochemistry-conference-in-september-2023-in-prague
Date: Sep 06, 2023
5. **International Conference on Pharmacognosy, Phytochemistry and Natural Products (ICPPNP) - London, United Kingdom**
Website URL: https://waset.org/pharmacognosy-phytochemistry-and-natural-products-conference-in-september-2023-in-london
Date: Sep 23, 2023
6. **International Conference on Medicinal Plants, Pharmacognosy, Phytochemistry and Natural Products (ICMPPPNP) - Rome, Italy**
Website URL: https://waset.org/medicinal-plants-pharmacognosy-phytochemistry-and-natural-products-conference-in-october-2023-in-rome
Date: Oct 18, 2023
7. **International Conference on Cholinergic Drugs and Phytochemistry (ICCDP) -** Istanbul, Turkey
Website URL: https://waset.org/cholinergic-drugs-and-phytochemistry-conference-in-october-2023-in-istanbul
Date: Oct 25, 2023
8. **International Conference on Pharmacognosy, Phytochemistry and Natural Products (ICPPNP) - Amsterdam, Netherlands**
Website URL: https://waset.org/pharmacognosy-phytochemistry-and-natural-products-conference-in-november-2023-in-amsterdam
Date: Nov 04, 2023
9. **International Conference on Pharmacognosy, Phytochemistry and Plant Products (ICPPPP) - Bangkok, Thailand**
Website URL: https://waset.org/pharmacognosy-phytochemistry-and-plant-products-conference-in-december-2023-in-bangkok
Date: Dec 16, 2023
10. **International Conference on Phytochemistry and Medicinal Plants (ICPMP) - Istanbul, Turkey**
Website URL: https://waset.org/phytochemistry-and-medicinal-plants-conference-in-december-2023-in-istanbul
Date: Dec 20, 2023

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