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Evaluation and Safety Study of Taraxacum Officinale Niosomes

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ABSTRACT

Phytosome process has been applied to many popular herbal extract including Gingko biloba, grape see, hawthorn, milk thistle, green tea and ginseng. The flavonoids and terpenoid components of these herbal extracts lend themselves quite well for the direct binding to Phosphatidylcholine. Phytosomes is produced by binding individual components of herbal extract to phosphatidylcholine, resulting in a dosage form that is better absorbed and thus, produces better result than the conventional herbal extract. Niosomes are one of the drug delivery system for targeting the specific site of the liver, brain etc. The objective of the present study was to encapsulate the drug in niosomal vesicles to reduce pulse entry of drug and to achieve sustained release when administered intravenously. The objective was further extended to target niosomes to liver schizontocidal stage of malarial parasites, thus to reduce dose related toxicity of the drug.

Literature revealed that the liver targeting agents like DMPC used in the vesicular formulation accumulate the maximum amount of drug in targeted cells, which would help in reducing the dose of the drug and it`s dose related toxicity. Certain of the water-soluble phyto-molecules (mainly flavonoids and other polyphenols) can be converted into lipid-friendly complexes, by reacting herbal extract owing to their enhanced capacity to cross the lipid-rich biomembranes and finally, reach the blood. They have improved pharmacokinetic and pharmacological parameters which are advantageous in the treatment of acute disease as well as in pharmaceutical and cosmetic compositions.

Keywords: DMPC, Phytosomes, Niosomes

Introduction

Phytosome is a patented process developed by Indena, a leading supplier of nutraceutical ingredients, to incorporate phospholipids into standardized extract and so vastly improve their absorption and utilization. It's a one kind of Phytosome formulation to deliver the herbal extracts.

The phytosomes of dandelion (Taraxacum officinale) were prepared with phosphotidylcholine in 2:1 and 1:1 ratio. These phyotosome were prepared and evaluated for quality control test and clearly mentioned in the work.

The phytosomes results were discussed in main experimental part of the thesis that are useful in liver detoxification as antioxidants. The herbal plant extract is generally used for heptic toxicity and phytosomes were formulated for detoxification of liver. The extract is used in skin disorders and treatment of cancer. Acne can be treated with plant extract phytosomes. The vesicular drug delivery system (phytosomes, ethiosomes, niosomes, microspheres and nano particles) are novel drug delivery system for many disorders. Phytosomes can better penetrate in lipid membrane and increase the availability of drug or phytoconstituents at particular site for

treatement of disease. Antioxidant properties can act as free radical scavenger for detoxification of many organs.

Keyword: Phytosomes, *Taraxacum officinale,*

1) MATERIALS AND METHODS:

Instruments used

- Soxhelt apparatus (Borosil Glass Works Ltd, Mumbai)
- Rotary evaporator (Buchi R-210, Switzerland)
- Digital balance-AX220 (Schimazdu, Japan)
- UV cabinet – 254 & 366 nm (Remi equipment pvt. Ltd, Mumbai)

Reagents and Chemicals: Analytical grade additives, reagents, and solvents, obtained from Merck Chemicals Pvt. Ltd, Qualigens Fine Chemicals Pvt. Ltd, and Sigma Chemical Company, U.S.A are. Used *Taraxacum officinale* leaf extracts in hexane, ethyl acetate, and methanol. The water was distilled using the MIlli-Q RO technique. Pre-coated TLC 60 F254 TLC aluminium silica gel plates were obtained from Merck Co., Mumbai.

2) Collection, extraction and preliminary phytochemical screening:

Collection of the plants: Leaves were obtained in the thick forest areas of Tirupathi, Tirupathi district, Andhra Pradesh. Fresh entire plants were harvested in February 2019, and the leaves were isolated from the plant, cut into small pieces, air dried, and cleaned of adherent foreign material. After that, they were mechanically pounded into a coarse powder (1kg). Dr. K. Madhava Chetty of professor in Botnay, Sri Venkateshwara University, Tirupati, 517 502, Andhra Pradesh had authenticated *Taraxacum officinale* was described, Analytical grade All chemicals and solvents from SD Fine Chemicals Pvt Ltd. (Sigma chemical company, U.S.A) were used.

Drying and storage: The leaves were obtained, cleaned, washed, and dried for three weeks in the shade at room temperature with intermittent shifting. And, using a mechanical grinder, they were powdered. The powders were sieved with 40 number sieves and placed in an airtight container for future use

Preparation of the plant extracts: Shade dry and coarsely powdered freshly harvested plant material Hexane, ethyl acetate, chloroform, methyl acetate ether and methanol were used to remove the dried powdered content (5 kg) in a Soxhlet apparatus. To acquire thick mass of the respective extract, the solvent was condensed under vacuum at a temperature of 40ºC expending a rotary evaporator (Buchi R-210, Switzerland). The extracts be situated extracted and processed in a desiccator in preparation for additional phytochemical and pharmacological research.

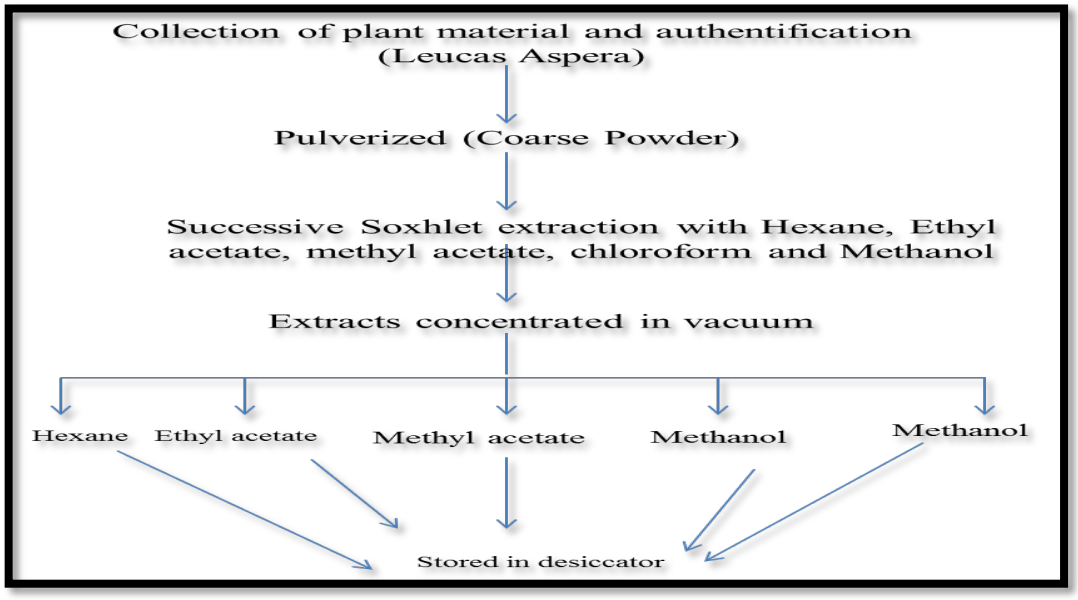

Figure 1: Schematic Representation of Extraction

Qualitative phytochemical evaluation: Hexane, chloroform, ethyl acetate, methyl acetate ether, and methanol of *Taraxacum officinale* were evaluated qualitatively using standard methods to identify different phytoconstituents. The extracts were subjected to the following chemical analyses, which were carried out according to standard methods stated in different books and journal publications.

A)Detection of Alkaloids: After stirring with a little quantity of weak hydrochloric acid, 50 mg extract free from solvent was filtered. The following alkaloidal reagents were used to test the filtrate.

a. **Wagner's Test**

Wagner's reagent few drops was supplementary to few mL of the dregs, from the assessment tube sides, leading to the reddish brown precipitate formation indicating test as positive.

b. Mayer's Test

Two drops of Mayer's element were applied to a few mL of filtered from the test tube's sides, resulting in a creamy white precipitate, indicating that the test was positive.

c. Dragendroff's Test

Dragendroff's reagent 1 or 2 mL is added to a few mL of filtrate appearance of prominent brown reddish precipitate indicates positive test.

d. **Hager's Test**

Add a small or 2 mL of Hager's reagent to some of those milliliter of filtrate. Positive results are indicated by a noticeable yellow precipitate.

B) Detection of Carbohydrates

Using 5 mL of water dissolve extract 100mg and filter and use this solution for below tests.

a. Molisch's Test

a – napthol two drops are auxiliary to 2 mL of filtrate. The assortmentbe there subjected to shaken and from the test tube sides Conc. H_2SO_4 1mL is added and by placing in the cold water and allowed to cool. The violet ring appearance at interface of liquids is due to occurrence of carbohydrates.

b. Fehling's Test

The appearance of red precipitate on adding both A and B solutions of Fehling's to 1 mL of filtrate indicate being there of sugars.

c. **Benedict's test**

Equal proportion of about 0.5 mL filtrate is mixed with Benedict's reagent and boil for 2 minutes on water bath. Prevalence of sugar is indicated by the appearance of characteristically coloured precipitate.

d. Barfoed's Test

Equal amount of about 1 mL filtrate is added with Barfoed's reagent and for 2 minutes it is boiled on water bath. If the precipitate obtained is red then it indicates sugar's presence.

C) Detection of Glycosides

For glycoside quantification, Strong HCl is used hydroxyl 50 mg of extract was by warming for 2 hours on water bathand then filtered, to perform following assay.

a. Borntrager's Test

About 3 mL ethyl acetate was mixed to 2 mL filtrate hydrolysate and agitated until the ethyl acetate layer split and a 10% ammonia solution is introduced into the solution. The anthroquinone glycosides presence leads to pink precipitate formation.

b. Legal's Test

To 20mg extract add pyridine and mix well to dissolve it. The aforesaid solution was turned alkaline by adding sodium nitro-prusside solution and 10 % NaOH solution. The presence of glycosides leads to formation of Pink precipitate.

D) Detection of Saponins

a.Froth Test or Foam test: 20mL solution is prepared by dissolving the extract in water and allowed to settle for 15 minutes in a graduated cylinder. The saponins presence in the solutions form's stable foam of 2 cm.

E) Detection of Triterpenoids and Phytosterols

a. Liebermann – Burchard's test

Dissolve extract using $C_4H_6O_3$, then boil and cool before adding 1 mL H_2SO_4 down the edge of the test tube. The prevalence of triterpenoids/steroids and their glycosides leads to appear red, pink, or violet tint at the liquid-liquid interface.

b. Salkowiski test

When a few droplets of conc H_2SO_4 are added to the ethyl acetate extract and mixed while standing, if bottom layer becomes red, indicates steroids presence, while the top layer becomes golden yellow, indicating the company of triterpenoids.

F) Detection of Phenolic and Tannins Compounds

a. Gelatin test

Add 2 mL of a 1 % gelatine solution containing 10% sodium chloride to a few mL aqueous extract solutions. White precipitate formation is due to phenolic compounds presences.

b. Test for ferric chloride

5% $Fecl_3$ few drops are added to 50 mg H_20 extract solution, violet, green, and blue colors formation indicate phenolic compounds presence.

c. Lead acetate test

To few mL aqueous extract solutions add 3 mL 10% Pb(C2H3O2)2, the phenolic compounds presence forms white bulky precipitate.

d. Alkaline reagents

The presence of Flavonoids causes the leaf extracts solution to glow yellow after being treated with 10% ammonium hydroxide.

e. Mg– HCl reduction or Shinoda test

Several millilitres of extract were dissolved in alcohol &a few pieces of magnesium turnings were added before adding HCl concentrated (drop wise). The presence of flavonol glycoside is inferred if any pink or crimson-red hue emerges.

f. Test for lignans

In investigation tube, 0.5mL of extract H_2O solution was added to 2mL of 2 % (V/V) furfur-aldehyde– red hue shows the presence of flavonoids.

i) Thin Layer Chromatography (TLC): TLC is a form of adsorption chromatography where adsorbent layer interact with the compounds in the sample and adsorbed them from the flow and thin adhered to a plate (stationary phase) and a solvent or solvent system (mobile phase).

Materials

- Silica gel G for TLC – Qualigens Co., Mumbai, India.
- Silica gel for CC (60-100 mesh size) - Qualigens Co., Mumbai, India.
- Basic alumina for CC – Acme Co., Mumbai, India.
- TLC plates (10 × 2cm, 20 × 5cm).
- TLC Sprayers – Borosil Co., Mumbai, India.
- Column chromatography columns.
- Chemical reagents (AR grade) were purchased from Qualigens Fine Chemicals Pvt. Ltd., Mumbai and Loba Chemical Company, Mumbai, India.
- Distilled water, Milli-Q.
- Rotary evaporator, Buchi, Switzerland. (267)

Preparation of Plates

100 g of silica gel-G is weighted and blended to a make a homologus solution of 200 mL distilled H_2O to make the slurry. The mixture solution is slowly poured on a TLC applicator and calibrated on flat glass plates of different diameters to a thickness of 0.25 mm (10 2, 10 5, 20 5, 20 10 cm). Coated plates are air dried then heated for 1hr upto 100-105 ^{0}c to prevent moisture, and then it is cooled, and stored in a dry environment. Before usage, the plates were heated for 10 minutes at 100° C to activate them.

The solvent systems and spray reagents listed below were employed to identify a few of the phytoconstituents identified on TLC [268].

Detection of Triterpenoids/ Steroids

- Used Solvent systems are
- water: methanol:Ethyl acetate: 4: 30 : 70
- water : methanol: Ethyl acetate: 8: 11: 81
- water: methanol: Ethyl acetate: 10: 15:75

Detection of Lignans

- Solvent systems used:
- ethyl acetate: Hexane: 1 :2
- ethyl acetate: Acetone: Hexane: 1:2: 7

Detection:

The TLC plates developed are elected using the vanillin-sulphuric acid reagent or methanolic sulphuric acid reagent (10%) sprayed and heat at 110ºC for 5 mins. Lignans appear as bluish-green spots. (269)

Vanillin eluting Reagents – Sulphuric acid reagent

Solution I: 5% ethanolic sulphuric acid (Universal reagent)

Solution II: 1% ethanolic vanillin

Solution I was sprayed over the formed TLC plate, then solution II is applied, later it is heated to 100ºc for about 5 to 10 minutes. Observe the Blue, blue-violet, or pink coloured patches are caused by steroid/triterpenoids and their glycosides. The VPA Reagent (Vanillin-Phosphoric Acid) is a mixture of vanillin and phosphoric acid (271)

1 gram vanillin in 100 mL phosphoric acid (Solution A) (50 %) 2 parts phosphoric acid (24%) and 8 parts ethanolic vanillic acid (solution B) (2%).

At 100 ºC for 10 mins the plate is heated after sprinkling the either solution A or B. The presence of triterpenoids/steroid glycosides is indicated by the red – violet color.

Antimony (III) chloride reaction reagent

20 % solution of antimony (III) chloride

The reagent was sprayed onto the TLC plate, which was then heated for 5-6 minutes at 100°C. Red – violet hue in visible light and red – violet, blue, and green fluorescence in UV at 365 nm suggest the presence of glycosides and their triterpenoids/ steroids.

3) ANTI BACTERIAL ACTIVITY

Medicinal plants are essential medical tools for a variety of illnesses, and they're also a source of new drug compounds for treating a variety of diseases. Plant origin natural products (Medicinal plants) have gained a lot of publicity in present days because of their broad range of biological properties, along with antimicrobial (against bacterial, fungal, and viral) activity. Antimicrobial therapy yielded significant progress, leading to an overly hopeful belief that infectious infections will be eradicated in the immediate future. In microbial assay, the following methods were used: a) cup plate or cylinder plate method; b) tube assay method or turbidimetric (serial dilution method). Antimicrobial screening was done in this sample using both the cup plate method as well as turbidimetric method. In the cup plate process, the antimicrobial compound propagates from the cup through a solidified agar substrate in a Petri dish or a plate to the point where the proliferation of added microorganisms is completely inhibited in a circular region or zone around the cavity containing a known amount of antimicrobial substance. The antimicrobial activity is assessed with a zone reader and represented as a millimeter zone of inhibition.

Test Organisms:

Eight bacterial species were used in this study. Bacteria were purchased from the National Array of Industrial Microorganisms (NCIM), which is based in Pune. The Bacteria were kept alive in the broth culture by inserting shakers in individual culture tubes for each species. Four of the eight Gram positive organisms are (Staphylococcus epidermidis, Bacillus megaterium, Streptococcus pneumonia, Bacillus pumillis,) and remaining four are Gram negative (Streptococcus pneumonia, Bacillus megaterium, Staphylococcus epidermidis, Bacillus pumillis) (Escherichia coli, Klebsiella pneumonia, Salmonella typhimurium, Pseudomonas aeruginosa).

Standardization of Micro-organisms:

In a 100 ml sterile media a loop of organisms was inoculated and cultured for Bacteria require 24 hours at 37°C and fungi require 48 hours at 27°C. Following incubation (24h/48h), 1 mL of the extremophile bacteria bouillon was poured to 9 mL of peptone water.

There were 100 l of dilutions ranging from 10-5 to 10-8 were spread on sterile nutritional agar (SDA) plates and maintained at 37°C and 27°C for 24 and 48 hours, respectively. In one millilitre of stock culture, the quantity of CFUs and microorganisms was counted and measured.

Preparation of Standard and Test Solutions

The DMSO was used to decompose the crisped extracts to make the sample compound stock solution at concentrations of 5 and 10 mg/ml, respectively. The reference criteria (Chloramphenicol and Nystatin) were formulated as stock solutions at a frequency of 1% in filtered water 0.6 mg/ml. By micro pipetting 0.05 ml stock solution into each cup, antimicrobial activity was determined [236].

a. Culture medium

For the anti-Microbial studies Nutrient Broth media was used.

b. Nutrient broth

Sodium chloride	- 0.5%
Beef extract	- 0.35%
Peptone	- 0.5%

37 g of the aforesaid materials were dissolved in H_2O (1000 ml). 7.2-7.4 pH was adjusted and the containers were sterilized using autoclaving at 15 smackers for 20 minutes.

c. Sterilization

The nutritional media, water, and other components were autoclaved for 20 minutes at 15 lbs/inch2. Pasteurization of glassware such as petri dishes, syringes, hollow test tubes, and pipettes is done in a dry heat oven at 160°C for one hour.

c. Nutrient agar Media

Agar	- 2.0%
Beef extract	- 0.3%
Peptone	- 0.5%
NaCl	- 0.5%
pH	- 7.2 to 7.4

The temperature of disinfected medium was slowed down to 40°C by cooling before being placed onto Petri dishes to a diameter of 6 mm. At room temperature, the media was enabled to solidify.

An agar transmission routine was castoff to test the in vitro antimicrobial potential of ethosomes against Candida albicans (ATCC 14057) in order to discover if the extraction process would affect the plant extract's antifungal efficacy. The bacterium was cultured on Sabouraud Dextrose Agar (SDA, Merck) for 48 hours at 25 degrees Celsius in order to conduct the experiment. SDA solution was added to a yeast suspension containing approximately 1.67 million CFU/mL of 0.9 % NaCl solution (25 % transmittance). A sterile whole puncher was used to generate wells with a diameter of 9 mm. This included each sample (phytosome suspension and gel), a positive control extract (drug solution), and a mixture of solvent and water that was used to dilute Efinaconazole and the formulations.

b. Evaluation of Antibacterial Activity by Cup Plate Method (IP. 2010)

In this assay method measuring precinct of embarrassment diameter of microbial decay around chambers (cups) comprising different dilutions of test compounds. In the agar medium, 4 cups of 6 mm diameter were prepared with the microbes and 0.1 ml of inoculum using a sterile borer. The spread plate technique was used to spread the cups on the agar plate. A micropipette was used to apply precisely calibrated (0.05 ml) solutions of every single deliberation and orientation canons to the dishware.

4. ACUTE ORAL TOXICITY STUDIES:

a. Guidelines 423- Acute Toxic Category Method

Guidelines for desperate oral noxiousness studies are controlled by the Association for Monetary Co-operation and Production (OECD). The OECD Guidelines for Chemical Testing was revised on a regular basis in light of technical advancements and changing evaluation methods. There are three instructions in total: 420, 423, and 425. This Guideline's acute toxic class approach is a stepwise technique that uses three animals of the same sex per phase. Depending on the animals' death and/or morbidity status, 2-4 measures may be required to make decisions on the test substance's acute toxicity. The procedure was widely tested in vivo against LD50 (lethal dose 50%) evidence collected from the literature, both nationally and globally, before it was introduced in 1996. The Guidance Document on AOTT - Acute Oral Toxicity Testing provides guidance on selecting the most suitable test procedure for a particular reason. The acute toxicity analysis was carried out to ensure the efficacy of the products, as well as to determine the Therapeutic Index (TI) and the lethal dose (LD50) value [238]. The immediate oral toxicity tests of ethyl acetate, methyl acetate, chloroform, hexane, and methanolic extracts of *Taraxacum officinale* on Swiss albino mice were carried out in this analysis according to the OECD guideline 420.

5. Anti-Inflammatory Activity

Different forms of reactions may arise when a researcher administers a chemical compound to a biological system, resulting in a sequence of dose-related responses. In certain instances, these reactions are desirable and beneficial, although there are a variety of unfavorable side effects. These may be dangerous to the patients or maybe not. Acute, sub-acute, and residual toxicity measures are among the categories of toxicity tests used by pharmaceutical companies in the development of new drugs. The drug is measured in a stepwise manner, with three animals of the same sex included in each step. The plant extract's immediate anti-inflammatory activity was tested using carrageenan-induced paw edema. This methodology has been widely employed for the development and evaluation of anti-inflammatory pharmaceuticals since the relative potency estimates derived from most treatments seem to represent clinical practice. This technique was selected for this study because carrageenan-induced edema is the supreme prevalent moderateuntried paradigm in the hunt for novel anti-inflammatory medicines.

Preparation of plant extract for dosing

Before administration, the plant extract utilized in the research was suspended in a 1 % (W/V) solution of Tween 80.

Experimental Animals

Acute toxic effect and anti-inflammatory tests were conducted on healthy Wistar albino rats ranging 120-150 gm. Animals were collected from the King Institute for Preventive Medicine in Guindy, Chennai, Tamil Nadu, India, and animals were housed in the Central Research Institute, Chennai. The animals were housed in polypropylene cages under customarystate of affairs of 12:12 (day/night cycles) at 22 ^{0}C part of buildingmalaise.

On healthy Wistar albino rats weighing 120-150 gm, anti-inflammatory and acute toxic impact tests were performed. The King Institute for Preventive Medicine in Chennai, Guindy of Tamil Nadu, India, provided the animals, which were kept at the CRI Institute in Chennai. The animals were kept in polypropylene barred enclosure at 22 ^{0}C room temperature under conventional 12:12 (day/night) settings. The animals were given a standard diet in the form of pellet and free access to tap water (Hindustan Lever Pvt. Ltd., Bangalore). Prior to the trials, the animals were kept in polypropylene cages for seven days to adapt to laboratory environments. Experiments were carried out in compliance with CPCSEA's latest recommendations in India. Both experimental studies were carried out in accordance with a procedure endorsed by the Institutional Animal Ethics Committee (Reg. No. 512/01/a/ CPCSEA) (Proposal No. 052/PHARMA / NCPA/ 2019).

Acute toxicity research experimental procedure

The assay was carried out in accordance with OECD recommendations 423. (OECD, 2001) Phase I surveillance began on day one, while Phase II inspection began 14 days later were the two stages of the experiment. The animals were maintained without food or water from 12 hours before to 3 hours after the oral administration. Pseudarthria Viscida root methanolic extract, 2000 mg/kg, was provided orally to the animals. The animals were scrutinized in lieu of toxicity signs for four hours after dosing, looking for autonomic and neurobehavioral changes. They were held under surveillance for 14 days, with body weight measurements taken on the 8th and 14th days. To eliminate the defects, the experiment was performed with a new group of animals. Appendix 12 contains a graphical flow map depicting the phases in the acute toxicity analysis.

In-vitro Security check for Anti-Inflammatory Properties

a) Method of denaturation of protein

Protein denaturation was used to denature polyherbal extracts at various concentrations. Bovine serum albumin (BSA) of 0.45 mL and 0.05 mL poly-herbal extract in varying proportions made up the reaction mixture (0.5 mL). At 370°C, the samples were incubated for half an hour [245]. After cooling the tests, phosphate buffer saline of about 2.5 mL of (pH 6.3) was added to each test tube. A spectrophotometer was used to calculate the turbidity of the samples at 660 nm. Excluding the extracts, 0.05 mL of distilled water was used as a control. The inhibitory proportion was calculated using the following formula [246].

$$\% \text{ inhibition} \quad \frac{AbsControl - Abstreated}{AbsControl} X\ 100$$

The protein was completely denatured in the control. The results were compared to acetylsalicylic acid (250mg/ml)-treated samples.

b) Edema in the paws caused by carrageenan

The rat-paw edema technique was hand-me-down to judge the acute anti-inflammatory response (111). All of the groups were given the test and standard medication formulations orally. Carrageenan (Normal saline about 0.1ml of 1 %) was administered stand inwindow boxseeming on apiece rat's left hind paw after one hour of test and standard administration [247].

The tibiotarsal junction of the hind feet was labelled so that appendagedimensions could be determined by way ofplummeting up to the mark into the mercury column of the plethysmograph. A plethysmometer was used to calculate the paw volume. The paw volume was calculated at intervals of 0 hours, 1 hour, 2 hours, 3 hours, and 4 hours. The volumes of

edema in the monitoring (Vc) and evaluation (Vt) care groups were calculated [248].

The paw volume %age increase was unwaveringexhausting the succeeding the formulae,

% Inhibition Vc – Vt / Vc × 100

Where Vt edema volume of the test;

Vc edema volume of control

c) Keloid scar exam on cotton pellets

The rats stood split into four teams, each of six animals, and the backs of their necks were bearded. Filamentshots weighing 10 mg be situated sanitized for around an hour before being injected hypodermically obsessed by rats with mild anaesthesia on both sides. All four groups were given oral therapy for seven days in a row after the cotton pellets were administered. Normal rats were given 10 mg/kg body weight of indomethacin sodium. On the eighth day, animals were sacrificed with ether, as well as pellets, which were sliced and recovered. The weight of the pills was calculated by drying them at 60°C. The weight of the granulomatous tissue produced was calculated as the discrepancy between the original and final weights. The %age of the calculation below was used to calculate granulomatous tissue development by blockage.

% Inhibition [1 – Wt / Wc] × 100

Where, granulation weight (Wt) - in treated group;

(Wc) -in the control group

Statistical Analysis:

MEAN SEM was used to represent the data. The acquired data were submitted to one-way variance analysis (ANOVA) and the Dennett non-test1.

Experimental Work

a) Test material

Ethyl acetate (PAEA) Hexane (PAHE), methanolic (PAME) extracts of *Taraxacum officinale* leaves

b) **Animals**

Female Swiss albino mice which are young fit adult nulliparous and non-pregnant, measuring 25-30g were provided by Mahaveer enterprises in Hyderabad at the start of the experiment. The animals are divided into three classes, each of six animals. The animals were chosen at random and held in their cages for 5 days before being dosed to enable them to acclimate to the lab conditions. The animals were held in individual cages made of sterile polypropylene. With a light-dark period of 12 hours, the room temperature and humidity were held at 25^0 C and 45-55 %, respectively. The animals were fed an available commercially standard pellet chow diet (Nutravet, Hyderabad) and given unlimited access to water.

c) Methodology

There are two kinds of as per the OECD, oral ingestion exposure studies are warranted guideline 420: limit assessment and main test. When the researcher has evidence that the investigation chemical is in the cards to be there harmless, i.e. harmful at levels below the regulatory limit concentrations, the limit test is more often utilized. Only the key examination should be done where there is little too little evidence regarding the toxicity of the test substance, or if the test material is expected to be hazardous. In this analysis, a limit test was carried out using the *Taraxacum officinale* as a nontoxic substance based on previous studies.

d) Procedure

All of the subjects were fasted overnight prior being measured, the dosage was determined based on body weight, and the checked sample was provided orally in a single dose of 2000mg/kg. Food was delayed for the following 3-4 hours after the sample was administered.

e) Observations

Animals were observed for harmful effects during the first 30 minutes after dose, then every 24 hours for the following 14 days (with particular care given during the first 4 hours). Remodelling in the eyes, nasal passages, skin, and other body parts hair, and behavioural patterns were all observed. Convulsions, Tremors, lethargy, salivation, sleep; shifts in body weight, coma, and mortality were all monitored.

RESULT AND DISCUSSION:

Alkaloids, carbohydrates, flavonoids, tannins, triterpenes, sterols, and saponins were found in the presence and Chloroform extract of *Taraxacum officinale* leaves. Table 1 summarizes the presence of carbohydrates, alkaloids, flavonoids, glycosides, tannins, and saponins in a methyl acetate extract of

Taraxacum officinale leaves, as well as the absence of various phytoconstituents. To identify the phyto constituents present in the extracts of the plant *Taraxacum officinale* (Wild) Connection, a qualitative phyto-chemical screening was performed.

Table 1: Nature of phytoconstituents present in hexane, ethanol, methanol, Chloroform and Methyl acetate extracts of *Taraxacum officinale* Leaves

Chemical Test	Hexane,	Ethanol	Methanol	Chloroform	Methyl acetate
Carbohydrates					
Molish's test	-	+	+	-	+
Fehling's test	-	-	-	+	+
Barfoed's test	-	+	-	-	+
Benedicts's test	-	+	+	-	-
Proteins & Amino acids					
Millions test	+	-	+	+	+
Biurette test	+	-	+	-	-
Ninhydrin test	+	-	-	+	+
Fats & fixed oils					
Saponification test	-	-	-	-	-

DETECTION OF SECONDARY METABOLITIES

Chemical Test	Hexane,	Ethanol	Methanol	Chloroform	Methyl acetate
Alkaloids					
Mayers test	-	+	-	+	+
Wagners test	-	+	-	+	+
Dragondroffs test	-	+	-	+	+
Hagers test	-	+	+	+	+
Steroids & terpenoid's					
Libermann-Burchard's Test	+	+	+	+	+
Salkowsky's Test	+	-	-	-	+
Phenolic compounds & Tannins					
$fecl_2$ Test	-	-	-	+	-
$Pb(C_2H_3O_2)_2$ Test	+	+	+	+	+
Bromine water test	+	-	+	-	-
Flavonoids					
Shinoda test	+	-	+	-	+
Alkaline reagent test	+	-	+	-	+
Saponin Glycosides					
Foam test	-	+	-	-	-
Glycosides					
Borntrager's Test	-	-	-	-	-
Keller-Killiani Test	+	-	-	+	+

+ Presence – Absence

Soxhlet extraction of *Taraxacum officinale*leaves yielded 6.45% hexane extract, 10.58% ethanol extract, and 8.94% methanol extract, as well as 8.12 p% chloroform extract and 6.12 % ethyl acetate extract, respectively. Terpenoids, phenols, sterols, lignans, carbohydrates, glycosides, alkaloids, tannins, flavonoids, and saponins were discovered in hexane, ethanol, methanol, chloroform, and methyl acetate extracts of *Taraxacum officinale*leaves. These lab tests may reveal the existence of triterpenoids in the plant. ursolic acid, Oleanolic acid and 3-sitosterol are all said to be present in the whole plant.

Nicotine, sterols, reducing sugars (galactose), and two new alkaloids (compound A, -sitosterol and -sitosterol , m.p. 61-2°,) (m.p. 183-4°), glucoside (230-1°), diterpenes (leucas perones B & A , Leucas perols A and B, isopimarane glycosides (leucas peroside (-)-chicanine, (75,85) and (7R,8R), myristargenol B, -licarin A,-2-(4 – allyl-2,6 – dimethoxyphenoxy) – 1-(4-hydroxy – 3-methoxyphenyl)propan – 1-ol,and machilin C, -2-(4 – allyl-2,6-dimethoxyphenoxy) – 1-(4-hydroxy – 3-meth). The major constituents of the 25 compounds detected from the leaf volatiles were u-farnesene (26.4 %), x-thujene (12.6%), and menthol (11.3 %).

The flower was found to contain ten compounds, the most prominent of which were amyl propionate (15.2%) and isoamyl propionate (14.4%). linoleic acid (48.11%), Palmitic acid (6.25%), oleic acid (42.07%), stearic acid (2.84%), and linolenic acid (48.11%) are all contained throughout the seed (0.65 %). 3-Sitosterol and ceryl alcohol were included in the unsaponifiable fraction. Novel aliphatic ketols (28-hydroxypentatriacontan-7-one, 7-hydroxydotriacontan-2-one), phenolic compounds (4-(24-hydroxy-1-oxo-5-n-propyltetracosanyl)-phenol), long-chain compounds (1-hydroxytetratriacontan-4-one, 32-methyltetratriacontan-8-ol), nonatriacontan Leucolactone (I) has been identified as 3, 3, 16c-dihydroxyoleanan-28-1,3-olide, which was isolated from the root of taraxcum officinale.

Table 2: Results of Total Phenolic content of *Taraxacum officinale* plant extract

Extracts	Total Phenolic content
Hexane extract	20.5±0.16
Ethanol extract	58.6±0.41
Methanol extract	30.4±1.2
Chlorofom extract	26.4±0.94
Methyl Acetate extract	20.7±1.1

Table 3: Results of Total terpenoids content of *Taraxacum officinale* plant extract

Extracts	Total Phenolic content
Hexane extract	30.5±0.34
Ethanol extract	68.6±0.67
Methanol extract	25.4±2.67
Chlorofom extract	30.4±2.4
Methyl Acetate extract	34.7±0.8

When compared to other prehistoric plant extracts, the leaves of *Taraxacum officinale* contained 80% of the total terpenoids, as did the fruits of C. carandas and L. maldivica, the seeds of E. cheiri, the entire plant of F. arabica, and the branches of W. somnifera. The seeds of C. speciosa contained 77% of total terpenoids, whereas the rhizome of B. lyceum contained 70%, the flower of R. indica contained 70%, and the fruit of D. peregrina contained 70%. All of the other plants produced a diverse range of terpenoids, ranging from 60% to 40%. With over 30,000 chemicals discovered, terpenoids were the most frequent and chemically fascinating group of secondary metabolites.

TLC studies:

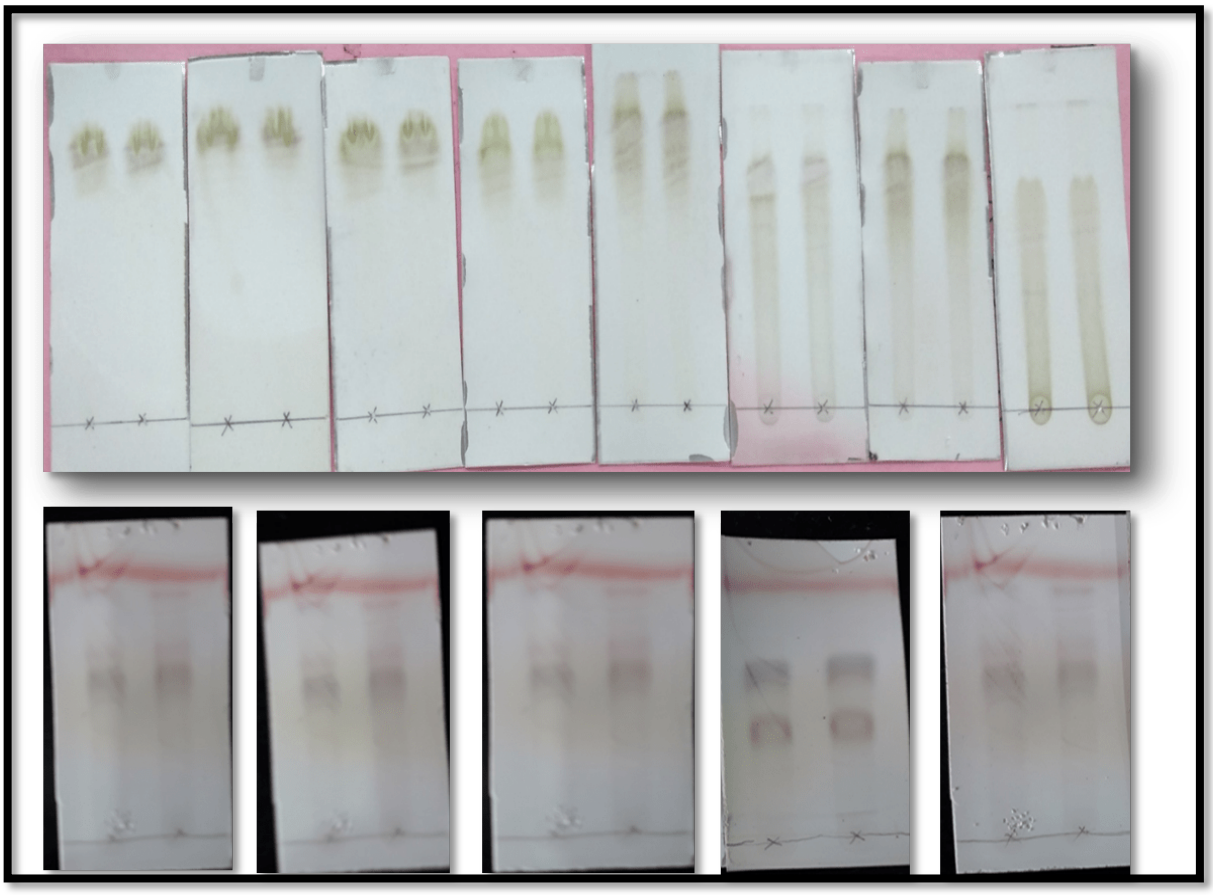

Figure 2: shows the Retention factor (Rf) for the four crude *Taraxacum officinale*.

Only the aqueous extract was found to lack a wide variety of phytochemicals. In addition, this helps to standardize the solvent solution for the separation of phytochemicals by providing essential information on polarity. Ethyl acetate and hexane extracts were found to have more phytochemicals than methanol and water extracts in this study. Colored samples were examined directly, while colourless samples were visualised using ultraviolet light (365 nm), iodine vapour, or by spraying the plates with $K_2Cr_2O_7$ in each of the three cases. As a last step, we determined the Rf values for each of the samples.

For the plant extracts, TLC profiling yielded a really outstanding result. It demonstrates the presence of a variety of plant metabolites in the extracts. Different solvent systems yield different Rf values for different phytochemicals. An insight of the polarity of the phytochemicals can be gained by looking at their Rf values, which can be used to pick a suitable solvent solution for column chromatography. Low-polarity compounds that have a high Rf value in less polar solvent systems have low polarity, while high-polarity compounds that have a low Rf value have high polarity. Mixtures of solvents with varying polarity in different proportions can be employed to separate the pure chemical from the plant extract. A plant extract's Rf values in different solvent systems should be taken into account while selecting the suitable solvent system. There are several phytochemicals in these plants, and their Rf values show their polarity, based on their TLC profiling in different solvent systems. This suggests that these plants contain a variety of phytochemicals. TLC profile of the plant under research will greatly benefit from knowledge of the polarity of the compound, the diversity of phytochemicals, and the choice of solvent system during isolation.

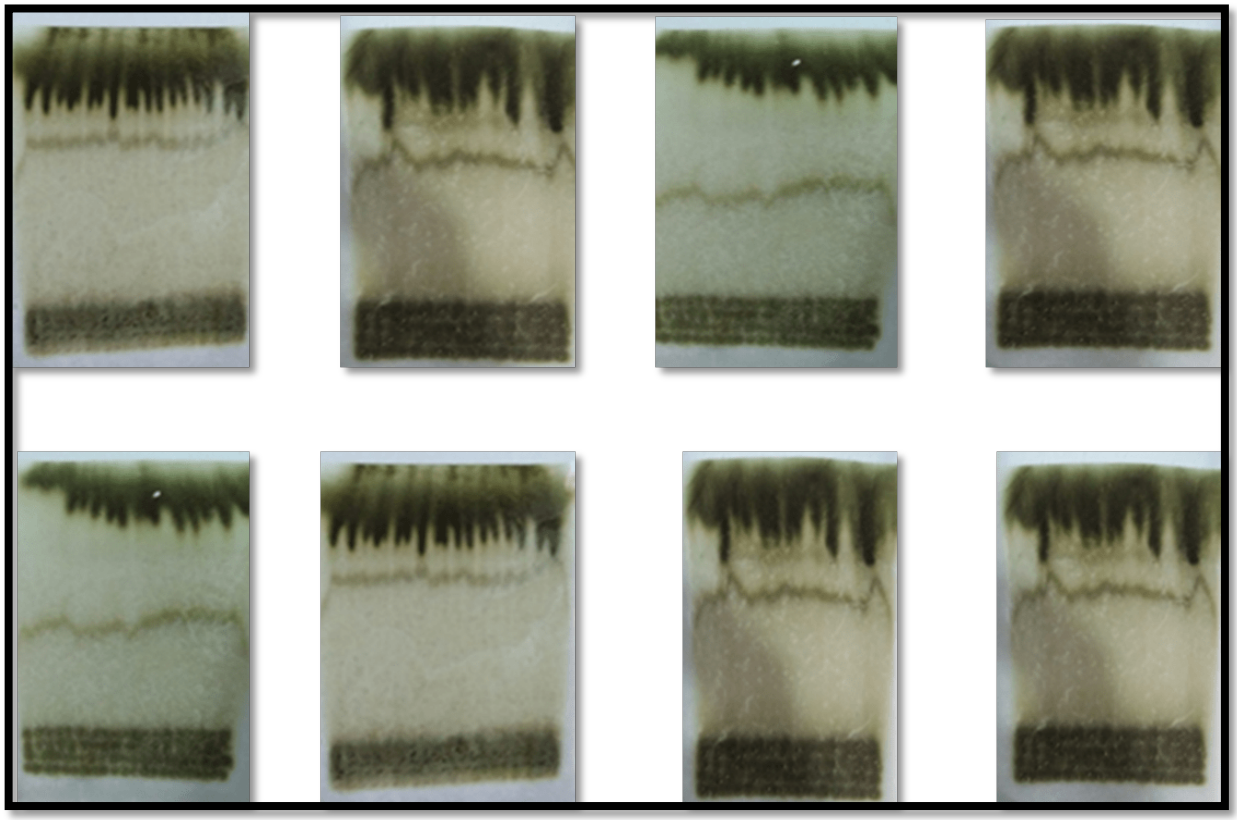

Figure 3: Preparative TLC Plates for separation of compounds

At room temperature, ethanol (3L, 14h) was used to extract pulverized leaf pieces (1.8 kg). The ethanol-soluble fractions subsequently filtered and concentrated at a lower pressure, yielding a greenish extract (57.0 g, 3.0% dry plant content), of which 55.0 g was divided using a chloroform: petroleum ether gradient, yielding 11 fractions LA1-LA9 (Chart 1).

LA4 (3.5g) and LA5 (1.3g) fractions were each treated with CC in a solution of 6% acetonitrile in methanol to yield 10 (LA4 (1-10) and 13 (LA5 (1-13) fractions, respectively.

To isolate compounds 1 and 2, fractions LA4 (7), LA4 (8), LA4 (9), LA5 (110), and LA5 (11) were combined and CCed in a 15 percent chloroform: ethyl acetate mixture (100mg) Fraction LA8 (3.8 g) was treated to CC with acetonitrile 1 to 3 percent in methanol to produce 15 fractions (LA7 (1-15)). To isolate compounds 3 (70 mg) and 4, fraction LA7 (10) (287.2 mg), CC in 15% chloroform: ethyl acetate was used (20mg).

The fractions LA9 (4) (400mg) and LA9 (2(5)) (1.3g) were subjected to CC separately utilizing an elution method of acetone 5 to 50% in ethyl acetate to obtain 15 (LA9 (2(4) a-o) and 10 (LA9 (2(4) a-j) fractions, respectively. To obtain 4 (LA9 (1-4) fractions, fraction LA9 (2) (2g) was exposed to CC in methanol: chloroform from 1 to 3 percent. The fractions LA9 (4) (400mg) and LA9 (2(5)) (1.3g) were subjected to CC separately utilizing an elution method of acetone 5 to 50% in ethyl acetate to obtain 15 (LA9 (2(4) a-o) and 10 (LA9 (2(4) a-j) fractions, respectively. The fractions were mixed (230 mg) and separated by CC with a 20% chloroform: ethyl acetate elution method.

Anti-microbial activity

The antibacterial activity of *Taraxacum officinale* ethanol extracts was shown to be superior to that of other extracts such as hexane, methanol, chloroform, and methyl acetate. The extracts had a greater impact on Gram negative bacteria than on Gram positive bacteria. In comparison to other extracts, hexane extract showed modest inhibitory zones against bacterial strains. A concentration of 150g/cup exhibited zone of inhibition against one Gram +ve (Bacillus megaterium) and two Gram –ve bacterial strains, with a maximal zone of inhibition of 9mm against Klebsiella pneumonia and Streptococcus pneumonia. This was against Gram +ve and Gram –ve bacteria. Hexane, Methanol, Chloroform, and methyl acetate extracts show modest inhibitory zones on the tested bacterial strains. For both P aeruginosa

and K pneumoniae, a maximum inhibition zone of 13 mm was found at a concentration of 1200 g/cup, while the maximum inhibition zone for Methanol extract was 13 mm for P aeruginosa and 12 mm for K pneumoniae.. Using a 1200 g/cup methanol concentration, the methanol extract had superior results against K pneumoniae, with inhibition zones up to 17mm. All of the information may be found in Table 04.

Table 4: Anti-microbial (Zone of inhibition) activity present in hexane, ethanol, methanol, Chloroform and Methyl acetate extracts of *Taraxacum officinale* Leaves

Name of the *Taraxacum officinale* Extracts	**Dose (µg/cup)**	**Zone of inhibition# (diameter in mm)**							
		Gram +ve				**Gram +ve**			
		B.p.	**B.m**	**S.e**	**S.p**	**E.c**	**P.a**	**K.p**	**S.t**
Methyl acetate	150	-	5	5	-	-	5	5	-
	300	5	6	6	5	5	6	6	5
	600	6	7	8	7	6	5	7	6
	1200	7	8	10	9	8	8	9	7
Chloroform	150	5	-	5	-	6	7	5	-
	300	7	5	7	6	8	9	7	6
	600	8	7	8	8	9	10	10	8
	1200	10	9	10	10	11	12	12	10
Methanol	150	6	5	-	-	-	6	5	5
	300	7	7	5	5	5	8	7	6
	600	9	9	8	7	7	10	9	8
	1200	10	11	10	9	10	13	12	10
Ethanol	150	5	5	5	7	5	7	6	6
	300	7	7	7	9	7	9	9	8
	600	10	9	10	11	9	12	11	10
	1200	13	12	12	13	12	14	14	12
Hexane	150	-	5	5	-	-	5	5	-
	300	5	6	6	5	5	6	6	5
	600	6	7	8	7	6	5	7	6
	1200	7	8	10	9	8	8	9	7
Chloromphenicol DMSO	10	14 -	17 -	16 -	15 -	15 -	14 -	17 -	16

B.p B pumillis, B.m B megaterium, S.e S epidermidis, S.p S pneumonia; E.c E coli, P.a P aeruginosa, K.p K pneumonia, S.t S typhimurium - No activity; # Values are the average of triplicate; Includes the Cup diameter (4mm)

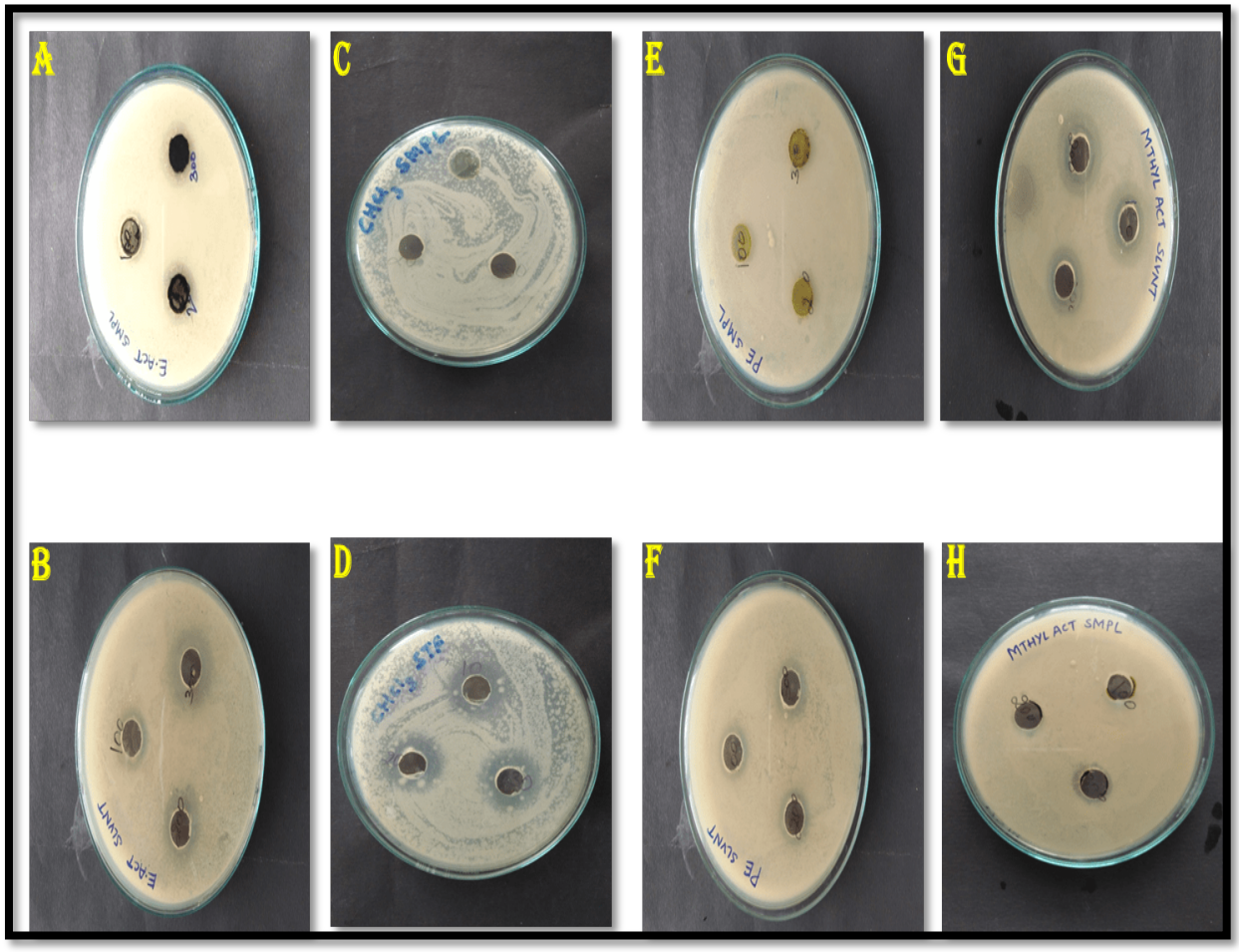

Figure 4: Anti-Microbial Activity of *Taraxacum officinale* A&B Zone of Inhibition of Ethanol, C&D Methanol, E&F Chloroform and G&H Methyl acetate

Plants form a resource of low molecular weight organic compounds that is largely untapped as a source of pharmaceuticals due to its immense array of secondary metabolites. New antimicrobial compounds have been developed from various resourses, including microorganisms, animals, and plants. Folk medicines are one of these tools. Systematic screening of these compounds may result in the identification of novel and more effective chemicals. In the last decade, plants and phytochemicals have become more important as potential sources of viral inhibitors.

Medicinal plants from compounds have been discovered in many places of the world that are active against microbes that cause human diseases. The results presented in this chapter clearly demonstrate that the extracts evaluated for antibacterial antimicrobial activity against a variety of diseases Bacteria with Gram +ve and Gram –ve were effective at concentrations of 150 g, 300 g, 600 g, and 1200 g per cup at the indicated concentrations (Table 04). Antimicrobials derived from plants have tremendous therapeutic potential, and they are successful for treating of infectious diseases and also have no adverse effects while avoiding many of the side effects that synthetic antimicrobials are known for. As a result, the antibacterial efficacy of such plants may be attributed to the presence of various phytochemical compounds. The findings that the plants have important antibacterial activity back up folkloric myths about their medicinal properties. Maybe people in and forest areas have been aware of the importance of plant products for centuries.

ACUTE ORAL TOXICITY STUDIES:

Table 5: Study of acute oral toxicity of extracts of *Taraxacum officinale* administered orally to mice.

Groups	Name of the Extract	Doses (mg/kg)	Sex	D/T	Mortality latency	Toxic symptoms
I	Hexane	2000	Both	0/6	----	Nil
II	Ethanol	2000	Both	0/6	----	Nil
III	Methanol	2000	Both	0/6	----	Nil
IV	Chloroform	2000	Both	0/6	----	Nil
V	Methyl acetate	2000	Both	0/6	----	Nil

D/T dead/treated rats; Nil no toxic symptoms observation; Mortality latency time to death (in hours) after oral administration.

To assess the safety of plant products and drugs for human use, toxicological evaluation in laboratory animals is performed to predict toxicity and allow for the selection of "healthy" doses. The lack of sufficient scientific evidence on the protection of *Taraxacum officinale*is often cited as a major barrier to its acceptance and use. Since the plant in this study was successfully classified as *Taraxacum officinale*, the findings cannot be extrapolated to this species. After the administration of extracts (Table 5), no toxic effects were found, suggesting that their lethal dose (LD_{50}) was greater than the test dose (2000 mg/kg). In acute oral toxicity tests on female albino mice, no signs of toxicity or mortality were found. The LD_{50} of *Taraxacum officinale* extracts in hexane, Methanol, Chloroform, methyl acetate, and ethanolic was found to be greater than the test dose (2000 mg/kg).There were no obvious signs of toxicity or mortality in the five extracts chosen. The findings showed that the extracts were non-toxic at a dosage of 2000 mg/kg. As a result, there is no LD_{50}, and all of the extracts tested are healthy and nontoxic.

Anti-Inflammatory Activity (*In-vivo* Screening methods)

a) Carrageenan induced paw edema test

The ethanolic extract of *Leucasaspera* leaves at different doses was administered in gum acacia suspension 18 hours and 2 hours before edema was induced by carrageenan injection, and the edema progression was followed.The area under the time-course (AUC) as total edoema reaction and the maximal paw edoema response. The maximum paw edoema response by rat paw acute inflammation was suppressed by indomethacin and alcoholic (methanolic) extract of *Taraxacum officinale* leaves Low dose: 100mg/50mg/kg, moderate dose: 200mg/100mg/kg, high dose: 400mg/200mg/kg When compared to the medication vehicle-administered control group, the total paw edema response (AUC) was inhibited by respectively over 6 hours. Table 06 and Figure3 summarise the findings.

Table 6: Effect of *Taraxacum officinale* on Carrageenan induced paw edema test

Time (hr)	Volume of edema (ml)				
	Control	IM (10mg/kg. P.O)	L. (250 mg/kg,p.o)	M. (500 mg/kg,p.o)	H. (900mg/kg,p.o)
1	0.53 ± 0.04	0.33 ±0.02 (38.4%)	0.40±0.03 (25%)*	0.33± 0.04 (40.3%)	0.30±0.02 (44.2%)
2	0.64 ± 0.01	0.36 ±0.03 (43.5%)*	0.45±0.02 (29%)	0.40±0.032 (37.0%)*	0.38±0.01 (40.3%)*
3	0.73 ± 0.05	0.32 ± 0.05 (58.6%)*	0.41±0.02 (44.0%)*	0.33±0.06 (52.0%)*	0.37±0.02 (53.3%)
4	0.66 ± 0.04	0.29 ± 0.03 (58.6%)*	0.43±0.02 (58.6%)	0.35±0.01 (58.6%)	0.34±0.03 (58.6%)

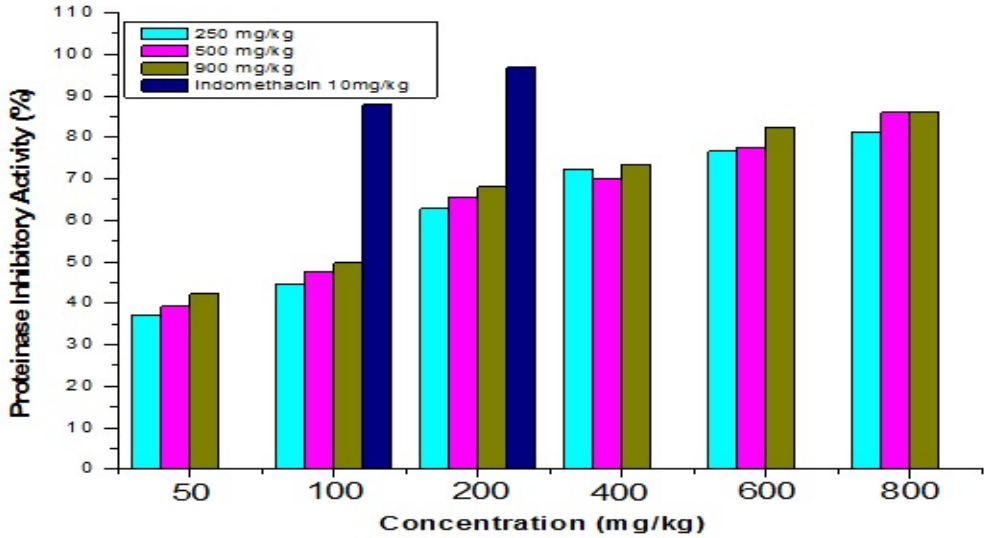

Figure 5:*Taraxacum officinale* ethanol Extract on proteinase inhibitory activity

Each statistic indicates the mean plus standard deviation. n6 groups were compared to the control group *P0.05 was regarded extremely significant. In comparison to the vehicle-treated group,

b) Cotton Pellet Granuloma Test

The effect of anti-inflammatory activity of the ethanolic extract of *Taraxacum officinale* leaves is shown in table 06 and figure 5.

Table 7: Effect of *Taraxacum officinale* on *Carrageenan induced paw edema test*

Treatment	Dry weight of granuloma
Control (CMC 0.5% 10ml/kg)	46.79 ± 1.64
Indomethacin (5mg/kg)	20.66 ± 1.67 (56.8%)*
Low dose (250mg/kg)	34.35 ± 2.29 (27.44%)*
Medium (500mg/kg)	30.29 ± 1.85 (35.0%)
High dose (900mg/kg)	24.49 ± 1.67 (47.94%)

The weight difference between a dry cotton pellet that has been dissected and a cotton pellet that has not been implanted. With n6 in each group, values are provided as mean standard error of the mean (SEM). In parenthesis, the proportion of granuloma formation suppression is shown in figure 6. When compared to the control group, *P 0.01 was found.

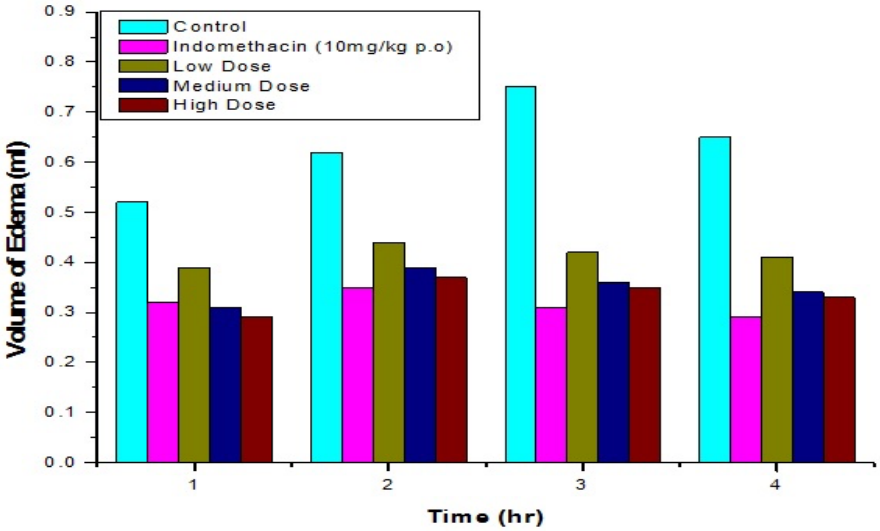

Figure 4:*Taraxacum officinale* ethanol Extract on Carrageenan-induced Rat Paw Edema

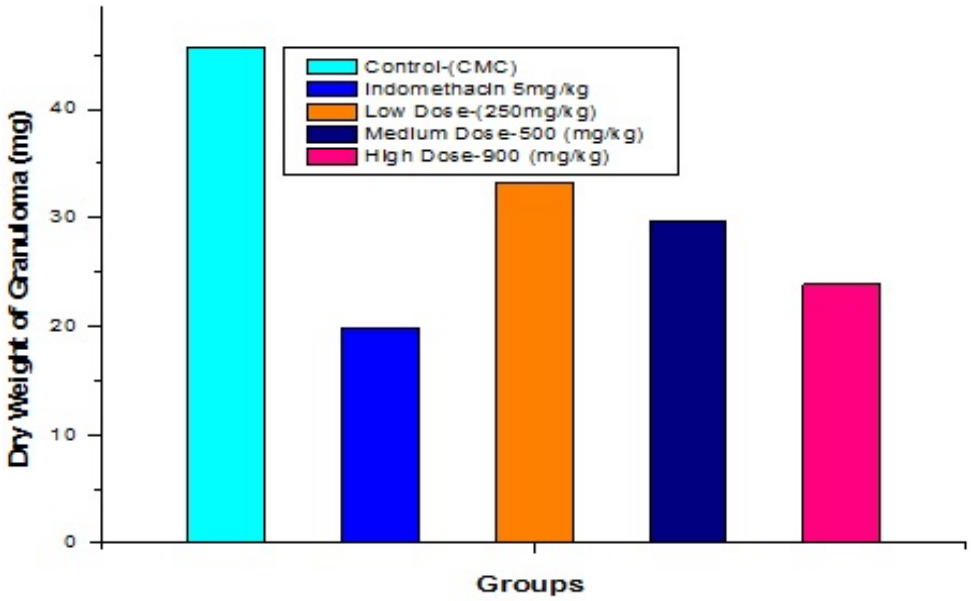

Figure 6: *Taraxacum officinale* ethanol Extract on Cotton Pellet Granuloma in Rats.

Conclusion:

Plant constituents have gained a lot of popularity and access to the global prescription market as a safer and more effective alternative to conventional drugs, which have a lot of negative and toxic side effects. Despite their enormous potential, phytoconstituents have drawbacks such as weak absorption and reduced solubility in both hydrophilic and lipophilic phases, the need for large doses, GI tract degradation, lack of uniformity, and inability to transportremedy to the targeted periodical.

The bioavailability of phytoconstituents such as flavonoids, terpenoids, and tannins, which have polyphenolic rings in their structure, has become a source of concern. Nanoparticle preparation, complexing with lipophilic carriers as liposomes or herbosomes/phytosomes, structural modifications, pro-drug delivery, and the addition of solubility and bioavailability enhancers.

Toxicological studies were performed to determined the safe level of PQP niosomal formulation which could be administered parenterally. Results of toxicity studies indicated that 5 fold decrease in toxicity of the niosomal formulation, when given intravenously in comparison to the conventional formulation. From these results we could concluded that the developed niosomal formulation was safe at preclinical stage with increase in LD_{50} and from histopatological studies it could be concluded that there were negligible necrosis at the targeting cells.

In an in vitro anti-inflammatory investigation, stress and inflammation were decreased. In vitro microbial investigations indicated the width of the zone of inhibition at the lowest inhibitory concentration against a particular bacterial organism, proving the antibacterial activity of the drug-loaded complex of phytosomes. An optimised complex of phytosomes loaded with medicine exhibited a considerable prolonged release profile and successfully encouraged topical administration, according to in vivo anti-psoriasis studies.

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