

## PHYTOCHEMICAL SCREENING, *INVITRO* ANTIOXIDANT, ANTIBACTERIAL ACTIVITIES ON *SKIMMIA LAUREOLA* LEAVES EXTRACT

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### ABSTRACT

**Background:** *Skimmialaureola* owned by the Rutaceae family is one of the plants that might use the Indian traditional treatment system in the Indian subcontinent and act against many diseases. So, this new research was carried out to scientifically confirm the various solvent fractions and separate the active fraction from the plant against antioxidants and bacterial and microbial growth.

**Objectives:** The main objective of the study involves the Phytochemical screening and research of *invitro* activities i.e. antioxidant and antibacterial from the extracts of the leaves part of the *Skimmialaureola* plant.

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**Methods:** Phytochemical screening was carried out of various extracts by the tests that involved it. *Invitro* antibacterial and antioxidant activities were examined of the plant extract through various methods DPPH, Metal chelating activity, Reducing power ability, Hydrogen peroxide scavenging activity, and Nitric oxide scavenging activity. After completion of the research, all the activity is shown graphically.

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**Results:** *Invitro* antioxidant activity shows that methanolic extract shows more antioxidant activity than other extracts. The antibacterial investigations show that the ethanolic extract of the plant removal was very compelling for bacteria *Streptococcus aureus*, *Bacillus subtilis*, and *Escherichia coli* at 25 µg/ml, 50 µg/ml, 75 µg/ml, 100 µg/ml, 200 µg/ml and 300 µg/ml individually.

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**Conclusion:** Auxiliary metabolites (phenol, flavonoid, tannin, and content) have been quantified. In antioxidant tests such as DPPH activity, the methanolic extract of the plant outperforms other extracts such as chloroform, petroleum ether, ethanol, and aqueous extract. The antibacterial investigations show that the ethanolic extract of the plant removed was very compelling for bacteria *Streptococcus aureus*, *Bacillus subtilis*, and *E. coli*. The current study may be useful in improving data regarding its distinguishing proof boundaries expected primarily in the method of the adequacy of homegrown medications in the current situation lacking administrative laws

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to control the nature of natural medications, as well as discovering antioxidants and antibacterial action.

**Keywords:** Pharmacognostical, medications, *Skimmialaureola*, antioxidants.

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## 1. Introduction

Herbal medicines may have food supplements that might be used as diet foods for good health. Many ingredients are included in food supplements such as minerals, vitamins, seasonings, amino acids and other supplements, antioxidants, macronutrients, tonics, and some herbal preparations also involve such as muslipak, chayawanprash, ashwagandhaleh, etc. All herbal plants extracts can show their effects on various properties such as antioxidants, antibacterial, anti-inflammatory, antihyperlipidemic, antidiabetic, antiarthritic, etc. This new progress is against antioxidants and control the strength of bacterial and microbial growth. (Kratchanova M., Denev P., Ciz M., 2010). Many herbal plants have various active constituents that help to inhibit various pharmacological activities such as polyphenols that can be reduced from those that can be reduced from what can be obtained from polyphenols. Natural herbs that provide effects on antihypertensive, antioxidant, antimutagenic, and antibacterial activity. Some of the natural antioxidants found in plant sources are culinary ingredients, spices, fruits found in plant sources are culinary ingredients, spices, fruit, found in plant sources are Culinary seasoning, spices, fruit, vegetables, and oil seed products. (Shahidi and Zhong, 2010). Many plants can show antibacterial activity or antifungal activity. They play an important role in bacteria and pathogens. Herbal medicines are used for large-scale treatment throughout India. Herbs are used in a wide distance as antibacterial and show some effects on disease while the problem of antibiotic resistance to overcome days day after day. The traditional medicine used is mostly by increasing people's demands as a comparison with confidential drugs. There has been much research progress in science about drugs and proven that in 20 years herbal medicines uses increased mostly. Many spices can show antibacterial or fungal activity and are used as secondary metabolites. Various parts of plants are useful for various bacterial diseases. Most of the investigations of researchers have that many herbs have microbial inhibiting properties. Scientists are looking for several herbal plants such as inhibiting the growth of *Streptococcus Bacillus*, *Aspergillusniger* and *E.coli*, and *Pseudomonas aeruginosa*. Herbal preparation can have

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**Comment [Dp16]:** Write it like this--- Research on medications has advanced significantly, and it has been demonstrated that the usage of herbal medicines has primarily increased over the past 20 years.

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an appropriate effect on the disease. Herbs are safe and effective for various biological properties. According to WHO, 20,000 plants can show positive effects and are used for treatment purposes. There are around 252 constituents found in the factory and used as drug preparation. 60% of drugs found in markets can be produced from natural sources and are used in various treatments of diseases. *Skimmialaureola* owned by the Rutaceae family is one of the plants that might use the Indian traditional treatment system in the Indian subcontinent. In the Himalayas, this plant is also known as Ner Patar and in Kamauni is used as a Dhoop or General. *Skimmialaureola* is very popular in Indian forests. *Skimmialaureola* is used as a bush that is planted as an ornamental plant. The leaves are used for burning to purify the air, they can also be eaten when cooked (Portners., 2017; Juss., 2009). The leaves give aromatic odors when crushed. White flowers develop small red berries (Juss., 2009). The distribution ranges from North China to North Himalayas. They are mainly planted in Vietnam (Portners., 2017; Juss., 2009). It is known that the demand for herbal medicine treatment for various diseases has increased, not only in India but globally. *Skimmialaureola* is a variety of four types of Evergreen bushes and small trees. This plant is naturally found in warm climates in Asia. Especially leaves have a cluster at the tip of the shoot, simple and lanceolate. And the margin must be smooth. Flowers have a solid panicle cluster, even though every flower is very small. The fruits must be a fleshy drupe that may contain one seed. All parts of the plant have a spicy aroma when crushed.



**Fig.1.**Plant of *Skimmialaureola*

## **2. Material and methodology**

### *2.1. Collection of plant material*

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The selected medicinal plants are collected from the high area of the Mandi district in Himachal Pradesh. Many medicinal plants can be found in forest areas that have a high height. The plants chosen can be largely cultivated in the forest area where the Mandi district is referred to as Devidarh. This experimental work was carried out at Abhilashi University Mandi in the Department of Pharmacognosy. Plant leaves are collected from the forest area in September 2020. After the collection of plants has been washed and dried thoroughly, it is dried under the shade which takes about a month. When the plant becomes dry rather than crushed into a mortar and converted into powder form. The shape of this plant powder can be preserved in a well-closed container.

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**Comment [Dp21]:** The plant powder doesn't have shape, size. Please correct the sentence

### 2.2. Preparation of plant material

Dried powder (10 gm) samples have been extracted out respectively with 100 ml of various variety of polarity solvents that turn into achievements such as water oil, ethanol, methanol, chloroform distilled water, and the use of soxhlet equipment. The extract has been filtered and evaporated in a hot water tub and weighed.

**Comment [Dp22]:** Refere it to draft newly -- Dried powder sample (10 gm) was extracted using 100 ml of the solvents which have different polarity including water oil, ethanol, methanol, chloroform, distilled water with the help of Soxhlet apparatus. The extract has been weighed after filtering and evaporating in a hot water bath.



Fig.2. Soxhlet assembly for extraction

### 2.3. Preliminary phytochemical analysis

Subjective examination of the leaves of *Skimmialaureola* showed the presence of different phytochemicals. More than one test was utilized on account of alkaloids, flavonoids, tannins, saponins, and steroids. Alkaloidal substances are detected in leaves. Examination showed the presence of flavonoids, saponins, phenolic compounds, and terpenoids in every one of the five concentrates including oil ether separate, ethyl acetic acid derivation removal, methanolic extricate, watery concentrate, and chloroform removal with their various tests (Table 1).

**Table 1.** Preliminary phytochemical analysis of various Extracts of the plant.

Sr. No.	Phytochemical Tests	Methanolic Extract	Chloroform Extract	Petroleum ether Extract	Ethyl acetate extract	Aqueous Extract	
1.	Test for alkaloids	Wagner's	+	+	+	-	-
		Mayer's	-	+	+	+	-
		Draggendorfs test	+	-	-	-	+
2.	Flavonoids	Shinoda test	+	-	+	+	+
3.	Tannins	Ferric Chloridetest	-	+	-	+	+
4.	Saponin	Foam test	+	+	-	-	-
5.	Triterpenoids	Salkowasky test	-	-	+	-	+
		Hishron test	+	-	-	+	+
6.	Amino acids	Ninhydrin Test	+	+	+	+	-
	Test for glycosides	Keller Killiani Test	-	+	-	-	+
		Legal take a look at	+	-	-	-	-
		Baljet test	-	+	+	+	+
8.	Test for proteins	Millon's Test	+	-	-	+	+
		Biuret Test	-	-	+	-	+

(+) indicates positive reaction, and (-) indicate negative reaction



**Figure 3.** Various test tubes carrying phytochemical screenings

#### *2.4. Pharmacognostical screening*

Pharmacognostical screening elaborates the macroscopical studies (shape, odor, taste, pattern, etc.), Microscopical studies, and standardization parameters from which the scientific information has been observed regarding the quality, quantity, and purity of the plant which is useful for a medicinal property of the plant.

#### *2.5. Standardization parameters*

The assurance of the abroad natural depend, misfortune on drying, debris values, extractive qualities, and so on. Gives a perfect idea about the exact qualities of unrefined medication under test, except for its large-scale morphological or cytomorphological, microscopical nature in the two its whole and its powder shape. These indicative elements permit the investigator to know the nature and capability of unrefined tablets. Unfamiliar natural matter, Debris values, Misfortune on drying, Extractive qualities, Powder investigation, and microscopy.

The powdered unrefined medication examination was intended to view and assess the extraordinary of regular pills for restorative value which is normally concentrated via old-style pharmacognostic studies. The validity of regular pills was affirmed by involving examination of their powder attributes.

#### *2.6. Phytochemical screening*

All the extracts had been subjected to phytochemical screening with the aid of dissolving them in respective solvents (1gm/ml) and with the aid of the usage of the unique reagents the

phytochemicals have been detected. Various tests are involved in it like Alkaloids, glycosides, Tannins, flavonoids, proteins, and amino acids.

### *2.7. Antioxidants activity*

Cell reinforcements are defined as materials that even at low mindfulness significantly defer or save you oxidation of simple oxidizable substrates. At the point when cell reinforcements respond with ROS or RNS, the cancer prevention agent is itself oftentimes changed over into a 'cancer prevention agent extremist'. Albeit the subsequent extremist has a limited capacity to respond with significant cell targets, it may as yet reason hurt (Buettner et al., 1993). The 'cell reinforcement extremist' wants to respond with each and every cancer prevention agent to bring the decreased potential and the reactivity further down.

Based on the response mechanisms concerned, antioxidant potential assays may be divided into most important groups: those primarily based on hydrogen transistor (HAT) reactions and others involving single electron transistor (SET) reactions (Huang et al., 2005; Prior et al., 2005). Since the hydrogen atom switch is a key step inside the radical chain, HAT-based total strategies are extra relevant to radical chain-breaking antioxidant capacity. In assessment, SET-based total assays contain one redox reaction in which the oxidant is likewise the explore for tracking the response. Single-electron transfer-based Assays contain components inside the reaction, i.e. The antioxidant and oxidant (also the probe), and comply with the relationship (Huang et al., 2005)

#### *2.7.1. DPPH Scavenging Activity*

The radical scavaging was investigated by the DPPH method by taking different concentrations of the extracts and make the extent to 100  $\mu$ L with methanol. Then 5 ml of 0.1M methanolic solution of DPPH was taken and incubated, absorbance was measured of the solution.  $IC_{50}$  (Inhibitory Concentration 50 %) was calculated from the extract and shown by the percent inhibition graph.

#### *2.7.2 Metal Chelating Activity*

The metal chelating activity was performed for antioxidant activity or prevention of such high-energy-free radicals. 2 mM of  $FeCl_2$  was added to the extracts in test tubes of triplicate determination. The reaction was initiated by way of including 0.2 ml of 5mM ferrozine solution.

The steel chelating hobby decided in EDTA equivalence evaluating the sample with the standard curve graph.

### 2.7.3. Nitric Oxide Scavenging Activity

Nitric Oxide Scavenging Activity of various plant extracts was carried out by adding sodium nitroprusside (10 mM) to the extracts along with Griess reagent (1 % sulphanilamide, 2 % H<sub>3</sub>PO<sub>4</sub>, and 0.1 % N(1-naphthyl) ethylene diamine dihydrochloride) to all the test tubes. The scavenging assay was calculated by the use of the subsequent equation.

### 2.7.4 Reducing Power Ability

For Reducing Power Ability methanolic extract was taken along with 2.5 mL of phosphate buffer (0.2 M, pH-6.6) and 2.5 mL of potassium ferricyanide (1 %) sequentially. Increased absorbance of the reaction mixture suggests increased lowering energy.

### 2.7.5 Hydrogen Peroxide Scavenging Activity

To carry out the Hydrogen Peroxide Scavenging Activity plant extract was taken with 600 µL of hydrogen peroxide approach to the plant pattern. The absorbance of hydrogen peroxide at 230 nm against the blank (phosphate buffer). The hydrogen peroxide scavenging assay is calculated by way of the subsequent formula.

## 2.8. Antibacterial assay

Similar to microorganisms, the flora is a biologically and chemically numerous resource. It is predicted that there are 250,000 to 500,000 species of vegetation on the Earth. Plants had been used as conventional drug treatments for the treatment of various illnesses at some stage in maximum of human records. The use of plant extracts as medicinal treatments received reputation within the overdue Nineties (Cowan., 1999). Plants are nevertheless an vital supply of drug treatments, mainly in developing international locations wherein the plant-primarily based conventional drug treatments are still used to fulfill the health-care desires (Salim., 2008).

### 2.8.1. Media Selection

During the complete research assignment styles of media have been used this is Nutrient Agar and Simple Agar. Nutrient Agar is the first-class culturing media for checking out micro-

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organisms because it provides nutrients for the growth of all kinds of microorganisms. 20 g of nutrient agar and 4g of easy agar turned into taken for the coaching of media.

#### 2.8.2. Compositions: a nutrient and simple agar

The nutrient agar media composition was yeast extract (3gm), beef extract (3gm), peptones (20gm), glucose monohydrates (20gm), agar (20gm), and sodium chloride (0.5g). the composition of simple agar media is agarose (20gm) and agaropectin (20gm).

#### 2.8.3. Preparation of media

20 grams of nutrient agar is changed into dissolved in 1 liter of distilled water in a conical flask and four grams of easy agar is also added and plugged into the flask and shaken to mix nicely. Then it is heated on the new plate stirrer to dissolve the media. The media and all glassware swabs had been sterilized by way of autoclaving under the 15psi and 121-degree centigrade temperature for 15 minutes in the autoclave. After this media changed into poured aseptically into Petri dishes in a laminar waft cabinet.

**Comment [Dp24]:** Construct it correctly

#### 2.8.4 Bacterial strains

The bacterial strain which is selected to carry out the work is *Escherichia coli*, *Streptococcus aureus*, and *Bacillus subtilis*.

#### 2.8.5. Procedure

Media that became coordinated and autoclaved became spread or unfurled on the Petri dishes in the laminar float cupboard. The Electric aficionado of the laminar coast cabinet was developed to become one to set the media and the pores are made in Petri dishes containing media by involving suggestions in a laminar float pantry. Then, at that point, the sanitized Q-tip changed plunged inside the refined water and afterward plunged inside the bacterial culture situated on the Petri dish containing media for you to streak lifestyle on the outer layer of supplement agar media of Petri dish consistently. One q-tip is utilized for handiest once streaking of 1 Petri dish then, at that point, disposed of (q-tip). Poured the new and bloodless water concentrates of verdure inside the well in the media of a Petri dish via a miniature pipette of 100 ml. After pouring all plates or Petri dishes have been hatched in an electric broiler or hatchery for around 24 hours at 37 degrees centigrade. And afterward, antibacterial movement becomes checked. The

quarter of hindrance become estimated with the guide of scale in mm following 24 hours the antibacterial interest had been doled out with regards to the area of restraint delivered through the plant extricates.

### 3. Results and Discussion

#### 3.1. Macroscopical Evaluation

##### 3.1.1. Macroscopical characters of *Skimmialaureola*

*Skimmialaureola* was collected and microscopic evaluation was carried out for the detection of the outer part of the plant like shape, odor, taste, pattern, texture, etc shown in Table 2.

**Table 2.** Morphological appearance

S.No.	Color	Dark Green
1	Taste	Pungent
2	Odour	Strong and Aromatic
3	Texture	Soft

Leaves: Mainly the leaves have clusters at the ends of the shoots, simple and lanceolate. The size of the leaves is around to be 6-21 cm long and 2-5 cm broad. And the margin must be smooth (Figure 3).

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Flowers: Flowers have dense panicle clusters, even though each flower is very small in size. The diameter of the flower is to be 6-15mm and have around 4-7 no. of petals in it (Figure 3).

Fruit: Fruit is to be a fleshy drupe that may contain a single seed. The color of the fruit is red to black and 6-12 mm in diameter (Figure 3).

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Seed: Ovoid to ellipsoid, reproductive structure membranous, reproductive structure copious; embryo straight; cotyledons rectangular to suborbicular, flattened, hypocotyl superior.

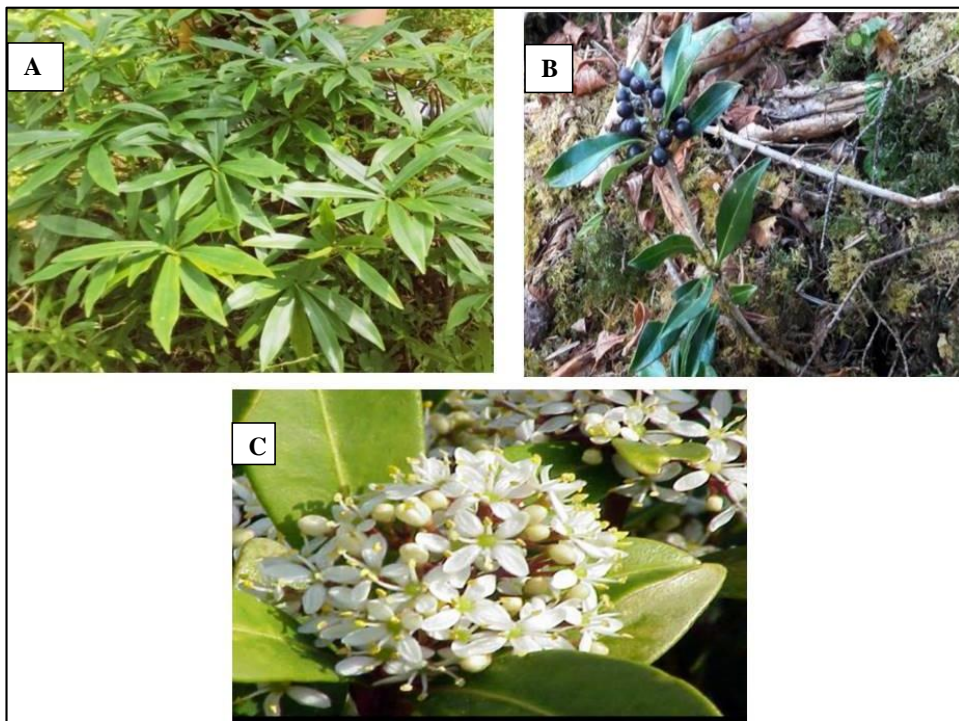


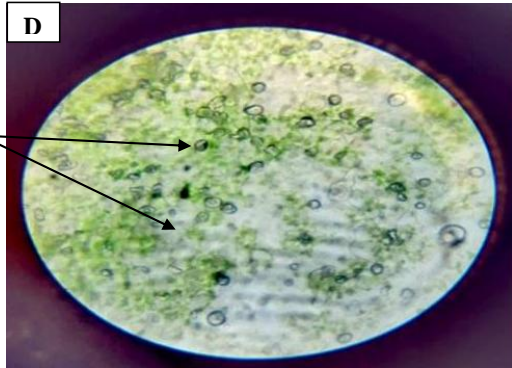
Figure 4. A) Leaves of *Skimmialaureola* B) Fruit of *Skimmialaureola* C) Flowers of *Skimmialaureola*

### 3.2. Microscopical Evaluation

#### 3.2.1 Quantitative microscopy

**Table3:** Various parameters of the plant.

Sr.No.	Parameter	Range
1.	Stomatal quantity	15-20
2.	Stomatal index	10-12
3.	Vien islet no.	11-13
4.	Vien let termination	12-15



Stomat



Vien islet no.

Figure 5. The Figure D shown the Stomatal quantity and Stomatal index, and E show the Vien islet no. and Vien let termination

### 3.2.2. Powder Microscopy

The powder of *Skimmialaureola* leaves is dark greenish in shading, fragrant scent, and is sharp, astringent in taste. Indicative person of leaves shows pieces of lignified and non-lignified epidermal cells. Silica stores, covering, basic trichomes, lignified strands, pieces of parenchyma cells loaded up with starch grain, earthy colored substance, scalariform vessel, sufficient measure of straightforward starch grains, oleoresin content. Indicative characters of the leaf show basic and covering trichomes, starch grain, a section of wavy parenchyma cell, lignified fiber, piece of the epidermal cell, stomata, saponin content, earthy colored substance, aleurone grain, annular

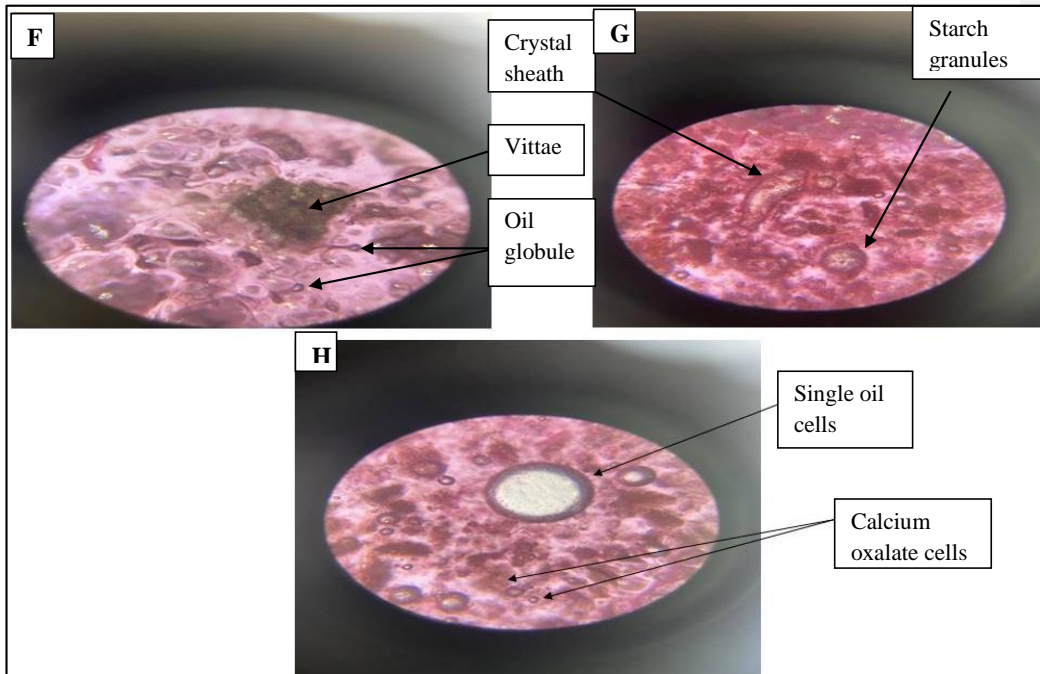


Figure 6. Figures (F, G, and H) show the presence of the various cells, grains, and globules in leaves.

### 3.3. Standardization Parameters

**Table 4.** Standardization parameters readings

Sr. NO.	Parameters	Values expressed as %
1.	Foreign organic matter	0.03 ± 0.170
2.	Ash values	
a.	Total ash	15.67 ± 0.090
b.	Acid insoluble ash	21.42 ± 0.064
c.	Water soluble ash	3.02 ± 0.052
3.	Loss on drying	6.30 ± 0.010

**Comment [Dp27]:** Values of it???....

4.	Extractive values	
a.	Water	20.88±0.361
b.	Ethanol	10.55± 0.234
c.	Methanol	21.47 ± 0.189
d.	Chloroform	8.23 ± 0.151
e.	Petroleum ether	30.22 ± 0.380

### 3.5. Powder analysis



Fig.7.Powdered drug of *Skimmialaureola*

The powdered crude drug analysis was aimed to study and also to assess the quality of herbal drugs for therapeutic value which are generally studied by classical pharmacognostical studies.

The authenticity of herbal drug was confirmed by comparison of their powder characteristics.

**Comment [Dp28]:** Correct the spelling

#### Table.5

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The reaction of various chemical reagents is tabulated in table below:

Powdered drug with reagent	Colour appeared in day light	Colour in UV light
Simple powder drug	Yellowish green	Greenish

Iodine with powder drug	Yellowish green	Dark green
IM HCL with powder drug	Pale green	Yellowish green
Acetic acid with Powder drug	Greenish	Dark green
Nitric acid with powder drug	Yellowish green	Dark brown
Sodium hydroxide with powder drug	Dark brown	Pale green

### 3.6. Antibacterial assay

**Table.6**

Zone of inhibition at various concentration of microorganisms.

Microorganisms	Zone of inhibition at various concentration					
	25µl	50µl	75µl	100µl	200µl	300µl
<i>Escherichia coli</i>	-	7mm	10mm	13mm	16mm	-
<i>Straptococcus aureus</i>	10mm	13mm	16mm	19mm	21mm	23mm
<i>Bacillus subtilis</i>	11mm	14mm	17mm	20mm	-	-

**Comment [Dp30]:** Genus species should in an italics

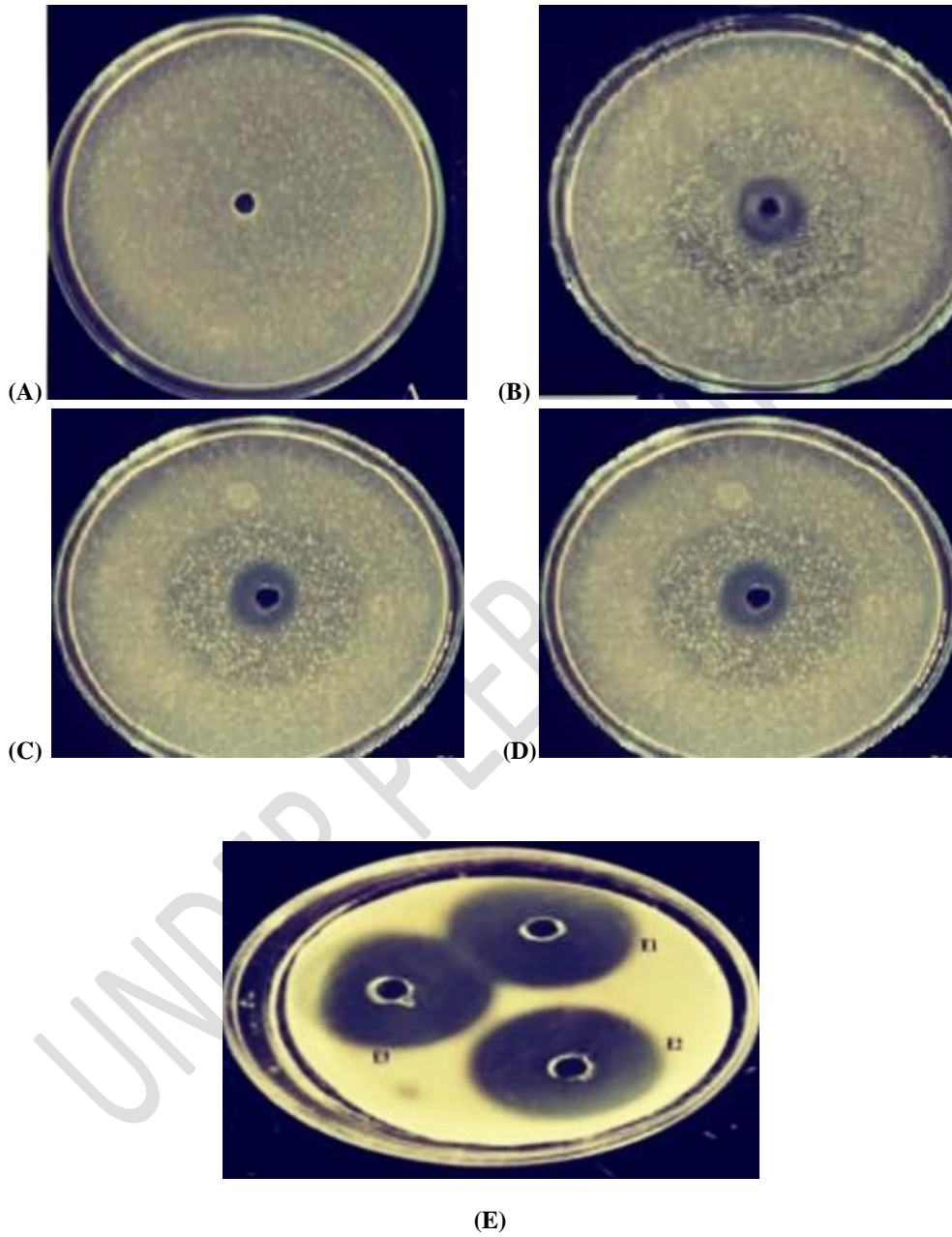


Fig.8. A,B,C,D,Ezone of inhibition at various concentration of Ethanolic extract of the plant *Skimmialaureola*



### 3.7. Antioxidant assay

#### 3.7.1. DPPH Scavenging Activity

Table.7

Showing DPPH activity on various concentration

Concentration	50	100	150	200	250	500
ASCORBIC ACID	47.28	49.98	54.55	62.95	73.56	82.56
PERN	4.55	5.45	7.56	10.56	15.56	19.54
CERN	6.54	8.58	9.05	11.54	19.56	27.45
EAERN	14.51	18.56	21.54	24.65	29.58	33.36
MERN	21.54	23.56	29.65	32.56	48.56	54.56
AERN	11.23	15.45	17.58	19.54	24.56	29.91

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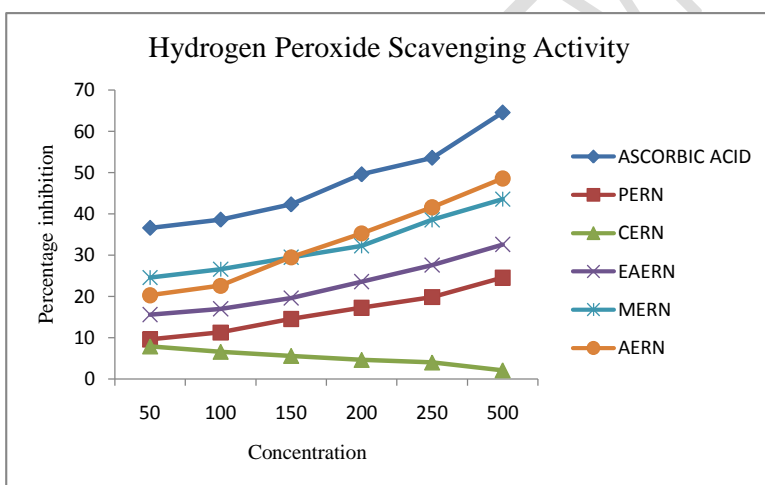


Fig.9. Graphically showing DPPH Scavenging activity of leaves extract of *Skimmialaureola*.

Ascorbic acid>MERN>EARN>AERN>CERN>PERN

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The DPPH activity searching movement of *Skimmialaureola* leaf is displayed in Figure 9. Among the concentrates tried, methanol extract has more effect and searching action of 50% at concentration of 500mg/ml.. Ethanol had a searching movement of 25% at concentration on 500mg/ml. Other extracts on different concentrations couldn't be reached even at higher fixations. In which petroleum ether show less effect on 19% at concentration of 500mg/ml.

3.7.2. Metal Chelating Activity

Table.8

Metal chelating activity on various concentrations.

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Concentration	50	100	150	200	250	500
EDTA	56.25	59.52	63.36	69.69	74.25	83.36
PERN	5.56	5.85	6.05	6.98	7.96	8.36
CERN	7.56	8.69	7.33	5.58	4.65	2.55
EAERN	20.23	22.23	25.56	29.45	34.54	38.65
MERN	29.56	31.55	37.74	39.56	42.25	49.55
AERN	25.55	26.45	29.56	34.56	39.54	46.56

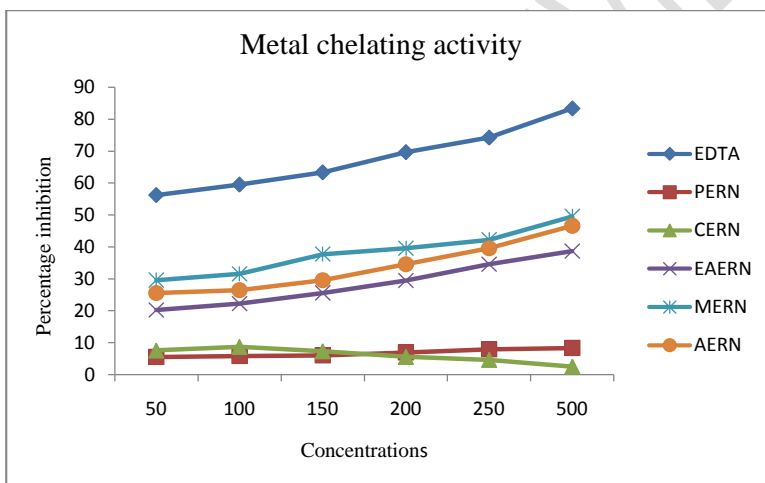


Fig.10.Graphically showing Metal Chelating activity of leaves extract of the plant *Skimmialaureola*.

EDTA>MERN> AERN>EARN> PERN> CERN

The antioxidative activity of *Skimmialaureola* leaf extracts in the metal chelating activity, and controlled by EDTA strategy is displayed in Figure 10. Methanol extract had an percentage 45% with concentration on 500mg/ml which showed a high metal chelating activity. Likewise with other dissolvable concentrates, half restraint of metal chelating activity couldn't be accomplished even at higher fixations. AERN showed an exceptionally metal chelating inhibitory action with an 20% inhibition with concentration 500 mg/ml. While chloroform extract show very less on given activity on 2% at concentration on 500mg/ml.

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Comment [Dp37]: Correct it grammatically

### 3.7.3. Nitric Oxide Scavenging Activity

**Table.9**

NO activity showing on various concentrations.

**Comment [Dp38]:** Mention the unit and check the font type

Concentration	50	100	150	200	250	500
ASCORBIC ACID	44.56	47.76	51.56	54.95	60.56	69.56
PERN	6.54	6.09	5.95	4.95	3.95	1.995
CERN	8.56	9.66	11.85	13.95	15.96	19.56
EAERN	12.55	13.06	15.85	18.56	21.96	27.56
MERN	29.56	31.54	36.85	42.95	45.96	57.55
AERN	13.96	27.45	34.45	35.95	38.56	47.56

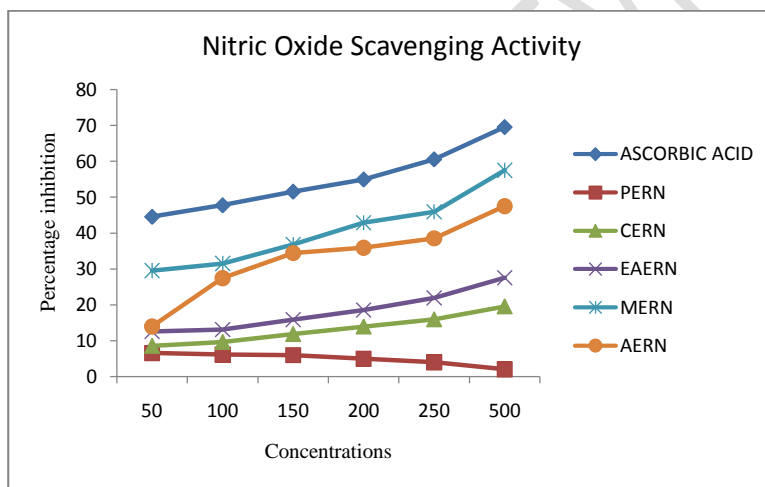


Fig.11. Graphically showing Nitric oxide Scavenging activity of Leaves extract of the plant *Skimmialaureola*.

Ascorbic acid>MERN> AERN>EARN> CERN> PERN

The Nitric oxide scavenging activity searching movement of *Skimmialaureola* leaf is displayed in Figure 11. Among the concentrates tried, methanol high effect and searching action of 50% at concentration of 500mg/ml. Aqueous extract had a searching movement of 40.1% at concentration on 500mg/ml. Other extracts on different concentrations couldn't be reached even at higher fixations. Where the petroleum ether show least effect on 1.995% on given concentrations.

**Comment [Dp39]:** Check all the red and blue lines underlined the words in the whole paper

### 3.7.4. Reducing Power Ability

**Table.10.**

Reducing power ability showing at different concentrations.

**Comment [Dp40]:** Same as above regarding unit and font type

Concentration	50	100	150	200	250	500
ASCORBIC ACID	1.054	1.145	1.445	1.596	1.725	1.925
PERN	0.036	0.045	0.059	0.074	0.085	0.096
CERN	0.049	0.056	0.068	0.075	0.081	0.085
EAERN	0.32	0.465	0.56	0.391	0.445	0.584
MERN	0.497	0.742	0.858	0.981	0.997	1.165
AERN	0.195	0.242	0.295	0.332	0.396	0.412

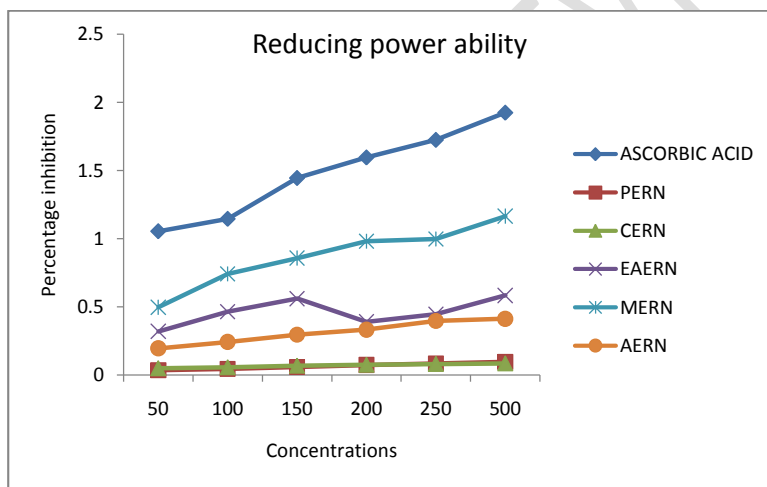


Fig.12. Graphically showing Reducing power ability activity of leaves of plant of *Skimmialaureola*.

Ascorbic acid>MERN> EARN> AERN>PERN>CERN

The reducing ability of *Skimmialaureola* leaves in various dissolvable concentrates is displayed in Figure 12. Methanol separate showed better lessening capacity when contrasted with ethanol, pet ether, chloroform and aqueous. Every one of the showed fixation subordinate movement. Methanol remove showed an increment in the percentage 2% with concentration at 50-500ml. Both aqueous and pet ether separates showed least diminishing capacities on given concentrations with 0.096% and 0.08%.

### 3.7.5. Hydrogen Peroxide Scavenging Activity

**Table.11**

H<sub>2</sub>O<sub>2</sub> activity showing on various concentrations.

Concentration	50	100	150	200	250	500
ASCORBIC ACID	36.56	38.56	42.26	49.56	53.56	64.55
PERN	9.56	11.25	14.55	17.25	19.85	24.56
CERN	7.86	6.56	5.56	4.59	3.96	2.06
EAERN	15.56	16.96	19.56	23.56	27.56	32.56
MERN	24.56	26.55	29.48	32.26	38.56	43.56
AERN	20.25	22.56	29.45	35.25	41.56	48.56

**Comment [Dp41]:** Must not be highlighted Font type, mention the unit

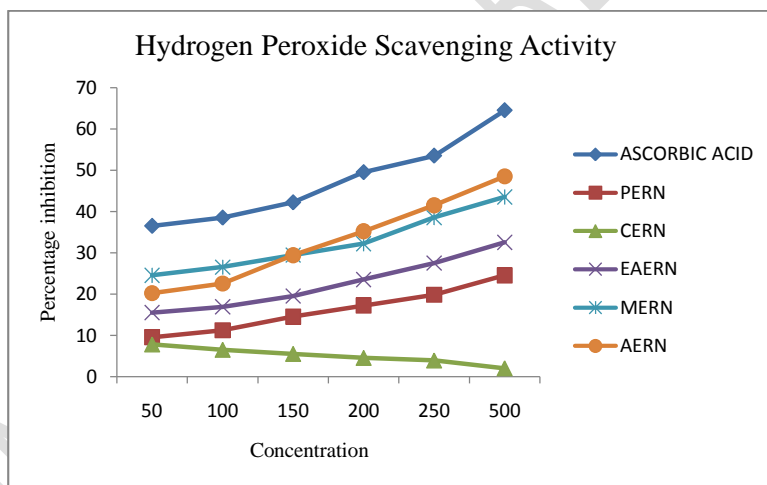


Fig.13.Graphically showing Hydrogen peroxide scavenging activity of leaves extract of *Skimmialaureola*.

Ascorbic acid>AERN>MERN> EARN> PERN>CERN

The Hydrogen peroxide scavenging activity of *Skimmialaureola* leaves in various dissolvable concentrates is displayed in Figure 13. Aqueous separate showed better lessening capacity when contrasted with ethanol, pet ether, chloroform and methanol. Every one of the showed fixation

subordinate movement. Methanol remove showed an increment in the percentage 35% with concentration at 500mg/ml. While chloroform showed least diminishing capacities on 2.06% on given concentration.

#### 4. Conclusion

The current study, titled "Phytochemical screening, *invitro* antioxidant, antibacterial properties on *Skimmialaureola* leaves extract," focuses on a plant that is widely available in India and is commonly used in the treatment of several ailments. There are currently no studies on the leaves of *Skimmialaureola*. As a result, in order to make the most of its potential, the current study focused on the leaves of this plant using a logical approach. The conformity of general features to the family *Skimmialaureola* was discovered after considering macroscopical components. Actinocytic stomata, multicellular uniseriate unbranched epidermal trichomes are discovered under the microscope. Stomatal number, stomatal file, vein islet number, vein end number are all quantitative microscopical exams. Microscopy of cell powder, fluorescence inspection of powder, and the results were also investigated. Sugar, alkaloids, flavonoids, protein and aminoacids, glycosides, and other compounds are discovered during preliminary phytochemical screening. Auxiliary metabolites (phenol, flavonoid, tannin, and content) have been quantified. In antioxidant tests such as DPPH activity, the methanolic extract of the plant outperforms other extracts such as chloroform, petroleum ether, ethanol, and aqueous extract. In this case, the pet.ether extract has a lower anti-activity impact. The methanolic extract has the greatest effect against activity in metal chelating, while the other extracts have a lesser effect. On different concentrations, nitric oxide activity and methanolic activity may have more impacts than other extracts. Methanolic extracts of hydrogen peroxide have a significant impact on antioxidant hydrogen peroxide activity. Reducing power ability to use power Aqueous extract has a stronger antioxidant action at varied doses. The plant's methanolic and aqueous extracts have antioxidant properties at varying doses, as shown visually. Antibacterial tests revealed that the ethanolic extract was particularly effective against *Straptococcus aureus*, *Bacillus subtilis*, and *E.coli* at concentrations of 25 g/ml, 50 g/ml, 75 g/ml, 100 g/ml, 200 g/ml, and 300 g/ml, respectively. The plant's methanolic extract may have a favourable effect against the several microorganisms we utilised to test its antibacterial activities. The effect appears to be 23mm at various concentrations, with a focus on the 300 g/ml number. In a concentration of 300 g/ml, *Escherichia coli* and *Bacillus subtilis* have no utility.

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### **Announcement of Contending Interest**

We wish to affirm that there are no known irreconcilable circumstances related with this distribution and there has been no huge monetary help for this work that might have affected its result.

### **Ethics approval and consent to participate**

When given the questionnaire form to collect ethnomedicinal knowledge, all participants gave their verbal prior informed consent.

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**Comment [Dp43]:** Numbering it one by one accordingly

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