



Evaluation of Therapeutic Efficacy of Ethanolic Extract of Terminalia Chebula as an Anti Bacterial and Anti Oxidant Agent

Gejalakshmi.S, Harikrishnan. N, Manojkumar.R, Marianancy. T, Pavithra.D, Priyadharshini.G, Rajamini.R

Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Dr.M.G.R.Educational and Research Institute, Velappanchavadi, Chennai-77

(Received: 04 February 2024

Revised: 11 March 2024

Accepted: 08 April 2024)

KEYWORDS

Terminalia chebula, invitro antioxidant, antibacterial, flavanoids

ABSTRACT:

Terminalia chebula, a phyto-medicinal herb extensively utilized in India, boasts remarkable healing properties. Its therapeutic potential encompasses a range of benefits, including antioxidant, anti-inflammatory, and antibacterial activities. Through phytochemical analysis, it has been revealed to contain various chemical constituents such as alkaloids, flavonoids, tannins, steroids, saponins, and glycosides. Moreover, studies evaluating its in vitro antioxidant and antibacterial activities have been conducted. Notably, *Terminalia chebula* has demonstrated a zone of inhibition against pathogens like *Klebsiella pneumoniae*, *Proteus mirabilis*, *Lactobacillus acidophilus*, and *Salmonella enterica*.

Introduction

Terminalia chebula is phyto-medicinal herb which has excellent healing power and widely utilized in India. It shows numerous therapeutic approaches especially antioxidant, anti-inflammatory and anti-bacterial activity present in the particular herb. It reveals various chemical constituents such as alkaloids, flavonoids, tannins, steroids, saponins and glycosides by performing phytochemical analysis. The evaluation of in vitro anti-oxidant and anti-bacterial activities has been performed [1]. *Terminalia chebula* has a zone of inhibition against *Klebsiella pneumoniae*, *Proteus mirabilis*, *Lactobacillus acidophilus* and *Salmonella enterica* [2]. The isolated constituents of *Terminalia chebula* was examined for the evaluation of anti-inflammatory activity by inhibiting the nitric oxide synthase (NOS) and cyclooxygenase-2 (COX-2) [3]. It has reported that the extraction performed with ethanol and acetone shows anti-oxidant activity. The isolated compounds of *Terminalia chebula* namely, casuarinin, chebulanin, chebulinic acid exhibits no antioxidant activity however it has anti-inflammatory activity [4]. The ethanolic extraction has been proven that it has high tannin rich source which is examined by liquid chromatography-mass spectroscopy. Regarding to binding affinity of tannins and targets has studied by in-

silico docking analysis. Corilagin is a compound isolated from *Terminalia chebula* has higher negative docking score results that it has good affinity related to anti-inflammatory activity [5]. The in vitro anti-oxidant activity is done by DPPH radical scavenging activity similarly anti-bacterial activity is done by Muller-Hinton Agar [6], [7]. Some of literature surveys says that it has less information on antioxidant and phytochemical analysis by the hot water extraction of *Terminalia chebula*. When pro-oxidants differ, then the antioxidant activity also differs [8]. Dried powdered leaves of *Terminalia chebula* has been extracted with hydro alcoholic solvent using maceration process and soxhlet extraction process for 48 hrs. The extracts were evaporated above their boiling points and stored in an air tight container free from any contamination until it was used. Finally, the percentage yields were calculated of the dried ethanolic extracts [9]. In this study, water-in-oil (w/o) emulsions were prepared by adding the aqueous phase to the oily phase under continuous agitation. The oily phase consisted of liquid paraffin oil and beeswax. The oily phase was heated up to $70 \pm 5^\circ\text{C}$. At the same time, the aqueous phase consisting of water (q.s.), borax and methyl paraben was heated to the same temperature before adding the *Terminalia chebula* extract. After that, the aqueous phase was added to the oil phase drop by drop. Stirring with the mechanical mixer was continued



at 2,000 rpm for about 15 min until all of the aqueous phase was added; a few drops of rose oil were added during this stirring time to give the formulation a pleasant fragrance [10].

Formulation was done and further evaluating various evaluation parameters such as Ph measurement, appearance, viscosity, acid value, saponification value, irritancy test, homogeneity, spreadability and determination of microbial examination. Washability was determined by rubbing the little amount of base on the hand and washing off with warm water without using soap should spread easily without too much drag and should not produce greater friction in the rubbing process. Spreadability was calculated using the spreadability apparatus made of wooden board with scale and two glass slides having two pans on both sides mounted on a pulley [11].

Oxidative stress is caused due to occurrence of free radicals and leads to cell and tissue damage. In mitochondria, reactive oxygen species is produced as an end product by the transportation of electrons which is lead up by oxidative stress. An imbalance between the detoxification and formation of pro-oxidants that induces the oxidative stress by inhibiting the anti-oxidant system or by formation of reactive oxygen species. Therefore, free radical scavenging, which guards against oxidative damage but has unfavourable side effects, is a common mechanism of action for synthetic anti-oxidant drugs. Consuming natural antioxidants from food supplements and conventional medications is an option and researchers are becoming more interested in phytotherapy by using medicinal herbs with antioxidant activity to prevent oxidative stress [12].

The antioxidant activity can be determined by various procedures. The most commonly used methods are inhibition of autoxidation of emulsion, Total radical trapping antioxidant parameter (TRAP), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and Oxygen radical absorbance capacity (ORAC). The ability of these techniques is measured by using a standard spectrometer measurement [13]. The spectroscopic method used is DPPH assay for free radical scavenging. It is fast, simple and effective method [14]. DPPH is a stable radical and it works on the principle that DPPH on accepting hydrogen atom from the antioxidant molecule, resulting in the colour change

from purple to yellow. This assay is used for the determination of parameters of antioxidant properties [15]. The results of the DPPH assay are expressed as IC₅₀ value. It is defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50% [16].

According to studies, 10 to 20% of herbal plants are used in the health care sector by treating various disorders. In the recent studies, medicinal herbs show anti-oxidant activity, anti -bacterial activity and anti-inflammatory activity. The active part of medicinal herb *Terminalia chebula* extracts obtained through soxhlet and maceration methods were subjected to preliminary antimicrobial screening against pathogenic microorganism [17]. The extraction of *Terminalia chebula* can be considered to be as equally potent as the most effective antibiotics, such as ciprofloxacin, gentamycin etc. It has more antibacterial potentiality. [18] Bacteria have the genetic ability to transmit and acquire resistance to drugs, utilized as a therapeutic agent. Research is used to understand the genetic mechanisms of resistance by controlling the use of antibiotic for the development of new drugs either naturally or synthetic [19]. The increase in antibiotic-resistance microorganisms has stimulated in research of novel antibacterial compounds. Various phyto-constituent have shown to exhibit antibacterial activity. They may also display the synergistic effects with existing antimicrobial drugs [20].

In this study, we examined that *Terminalia chebula* has various activities which is essential to act against various disorders. Phytochemical analysis has been performed to determine the chemical constituent present in the *Terminalia chebula*. It is more effective as it acts as anti-oxidant and anti-bacterial activity. These activities have been proved so that *Terminalia chebula* is utilized in various formulation. In this study we formulated *Terminalia chebula* as ointment and evaluated its parameters.

1. Literature Review

Singh AK, Kumar P, Rajput VD, et al.(2023).evaluated based on hyperglycemia diabetes which leads to diseases like retinopathy, nephropathy, neuropathy and cardiovascular disease. The polyherbal extract was formed by mixing equal parts of *Terminalia chebula*



Retz. (TC), *Terminalia bellerica* Roxb. (TB), *Berberis aristata* DC. (BA), *Nyctanthes arbostratis* L. (NA), *Premna integrifolia* L. (PI), and *Andrographis paniculata* (AP). By analysing polyherbal extract findings concluded that it consists of phytochemicals such as glycosides, flavonoids, alkaloids, tannins, phytosterols, and saponins. By using in vitro investigations antioxidant and anti-inflammatory, the study's objectives were to generate an ethanolic extract of polyherbal extract using the cold maceration technique, identify bioactive compounds via gas chromatography-mass spectrometry (GC-MS) analysis, and assess biological responses. Polyherbal extract included 35 phytochemicals in total, 22 of which were bioactive components that were recovered in a good quantity. A few novel ones are identified, including 2-buten-1-ol, 2-ethyl-4-(2, 2, 3-trimethyl-3-cyclopenten-1-yl) (17.22%), 1, 2, 5, 6-tetrahydrobenzonitrile (4.26%), 4-piperidinamine, 2, 2, 6, 6-tetramethyl-(0.07%), undecanoic acid, 5-chloro-, and chloromethyl ester (0.41%). EC₅₀ values of 392.143 µg/ml were used to measure antioxidant activity. The obtained value was similar to the standard value by using DPPH. This study resulted in bioactive compounds that are used to examine biological activity. Concluding that in-silico research like docking are been conducted to know more function on diabetes receptors.

Na Takuathung M, Wongnoppavich A, Jaijoy K, et al. (2023) studied that *Terminalia chebula* was used to treat disorders including skin diseases. The main desire of this study is to evaluate the fruit of *Terminalia chebula* for its antioxidant effect and antitumorigenic activity. Furthermore, they conducted Folin-Ciocalteu, DPPH and ferric reducing antioxidant power (FRAP) tests to determine the total phenolic content and antioxidant activity. To evaluate the extract's ability to reduce intracellular reactive oxygen species (ROS) in U-937 human monocytic cell lines, 2,7-dichlorodihydrofluorescein diacetate assay was performed. To determine the extracts primary phenolic components High-performance liquid chromatography was carried out. For statistical analysis ANOVA test was used. In vitro, T chebula extract efficiently scavenged antioxidants and decreased intracellular ROS. Using the DPPH and FRAP tests, the extract's IC₅₀ values were 2.39 ± 0.17 µg/mL and 109.0 ± 14.5 µg/mL, respectively. Therefore, the extract from *Terminalia chebula* decreased

intracellular ROS. As a conclusion, to treat oxidative stress related disorders including skin tumours, extract from *Terminalia chebula* plays a major role.

Bhujbal A, Mohite M, Alhat S, et al. (2023) Invented a polyherbal cream which consists of extracts from *Terminalia chebula*, *Cassia tora*, *Ocimum sanctum*, and *Cynodon dactylon*. This cream is used to treat psoriasis, chronic autoimmune skin condition marked by scaly patches on the skin. Following formulation, the evaluation test is carried out for the physical and chemical properties of the cream. They determined that the anti-inflammatory and antioxidant qualities of the cream is strong, by the study results. However, the creams anti-inflammatory property was determined by the protein denaturation method, in vitro cell viability assay and anti-microbial study. These findings imply that the polyherbal cream containing extracts of *Terminalia chebula*, *Cassia tora*, *Ocimum sanctum*, and *Cynodon dactylon* could be a useful adjunct or substitute treatment for psoriasis. As a result, they concluded that additional clinical trials are required to confirm the creams long term safety and effectiveness.

Kumar S, Chauhan N, Tyagi B, et al. (2023) analysed that the aim of the study was to determine the bioactive components, therapeutic application and antioxidant capacity of different extracts of medicinal plants. They also determined the Total phenolic extract (TPC), Total flavonoid contents (TFC) and antioxidant capacity by the Folin-ciocalteau reagent, aluminium chloride calorimetric assay, DPPH, and FRAP assay. The extracts of *Terminalia chebula*, *Aloe vera*, and *Curcuma longa* in methanolic form had the greatest total phenolic content values ($p < 0.05$). When measured against other extracts, the total antioxidant capacity of the *Ocimum tenuiflorum* and *Aloe vera* extract was the greatest ($p < 0.05$). *Zizyphus zuzube*'s aqueous extract had the lowest FRAP value. Of the medicinal plant species being studied for possible therapeutic application, phenolic chemicals are the main source of the antioxidant activity. All things considered; the current study showed that the chosen plants are possible producers of bioactive compounds that might scavenge free radicals.

Sultan MT, Anwar MJ, Imran M, et al. (2023) defined that the current market trend changed as long-term effects involving dietary and lifestyle modifications. The research in the chemical and pharmaceutical sciences lead to the invention of medications which later on raised



concerns about their safety and toxicology. Researchers examined plants that were formerly utilised in Ayurvedic and Chinese medicine in order to confirm their traditional uses. The most commonly used herbal is myrobalan (*Terminalia chebula*) which is used to treat various health issues and it is also known as the "king of medicinal plants" in Ayurveda. The phytochemical profile revealed by the current review contains rutin, corilagin, chebulagic acid, phenolic acids, and casuarinin. Its medical uses are associated with phytochemistry, and numerous investigations have confirmed its antibacterial, anti-inflammatory, hypoglycemic, antioxidant, and digestive tonic properties. This article also highlights the conceptualised framework for effectiveness against neurological illnesses, cancer resurgence, immunological dysfunction, and cardiovascular disorders. Finally, a thorough analysis of its possible place in the current functional food market is presented, along with its potential future uses.

Thoithoisana Devi S, Devika Chanu K, et al. (2023) the author summarised in this article they used a well-known medicinal plant that grows commonly in Manipur. It is locally known as Manahei (*Terminalia chebula*). They used it as a mild laxative, anti-inflammatory and treatment for several diseases. The hydroalcoholic extract from four *Terminalia chebula* fruit samples from various locations in Manipur was analysed by Gas chromatography-mass spectroscopy (GC-MS) and High performance thin-layer chromatography (HPTLC). The extract's chemical constituents were assessed, and its anticancer activity against the colon cancer cell HCT 116 was assessed. The findings of the GC-MS analysis showed that there was a considerable variance in the extract's principal compound proportion and composition. All extracts showed gallic acid and ellagic acid with HPTLC. Gallic acid concentration was highest in TCH, while ellagic acid content was highest in TYH. The cytotoxic impact of TYH in HCT 116 cells was validated by the LDH test. After 48 hours of therapy, it was also discovered that TYH caused caspase-dependent apoptosis in HCT 116 cells. Their research sheds light on the variety of *T. chebula* found in Manipur as well as its possible anti-colorectal properties.

Sultan MT, Anwar MJ, Imran M, et al. (2023) defined that the current market trend changed as long-term effects involving dietary and lifestyle modifications. The research in the chemical and pharmaceutical sciences

lead to the invention of medications which later on raised concerns about their safety and toxicology. Researchers examined plants that were formerly utilised in Ayurvedic and Chinese medicine in order to confirm their traditional uses. The most commonly used herbal is myrobalan (*Terminalia chebula*) which is used to treat various health issues and it is also known as the "king of medicinal plants" in Ayurveda. The phytochemical profile revealed by the current review contains rutin, corilagin, chebulagic acid, phenolic acids, and casuarinin. Its medical uses are associated with phytochemistry, and numerous investigations have confirmed its antibacterial, anti-inflammatory, hypoglycemic, antioxidant, and digestive tonic properties. This article also highlights the conceptualised framework for effectiveness against neurological illnesses, cancer resurgence, immunological dysfunction, and cardiovascular disorders. Finally, a thorough analysis of its possible place in the current functional food market is presented, along with its potential future uses.

Thoithoisana Devi S, Devika Chanu K, et al. (2023) the author summarised in this article they used a well-known medicinal plant that grows commonly in Manipur. It is locally known as Manahei (*Terminalia chebula*). They used it as a mild laxative, anti-inflammatory and treatment for several diseases. The hydroalcoholic extract from four *Terminalia chebula* fruit samples from various locations in Manipur was analysed by Gas chromatography-mass spectroscopy (GC-MS) and High performance thin-layer chromatography (HPTLC). The extract's chemical constituents were assessed, and its anticancer activity against the colon cancer cell HCT 116 was assessed. The findings of the GC-MS analysis showed that there was a considerable variance in the extract's principal compound proportion and composition. All extracts showed gallic acid and ellagic acid with HPTLC. Gallic acid concentration was highest in TCH, while ellagic acid content was highest in TYH. The cytotoxic impact of TYH in HCT 116 cells was validated by the LDH test. After 48 hours of therapy, it was also discovered that TYH caused caspase-dependent apoptosis in HCT 116 cells. Their research sheds light on the variety of *T. chebula* found in Manipur as well as its possible anti-colorectal properties.

Randhawa M, Meyer T, Sachdev M, et al. (2021) examined that the aim of the study is to find a long-lasting natural antioxidant to prevent and treat skin



damage caused by pollution in the environment. They implemented it by examining an 8-week clinical trial for the antioxidant and anti-inflammatory activity of a standardised fruit extract from *Terminalia chebula*. They used subjects who had normal to dry skin and had lived in a high-pollution city for the previous five years to determine whether the formulation containing 1% standardised *Terminalia chebula* fruit extract had a significant positive impact on the visible condition and appearance of the skin when compared to a placebo. When keratinocytes were treated with the fruit extract before being stressed by urban dust, it prevented the release of inflammatory cytokines like IL-6 and IL-8, prevented the increase of intracellular ROS, and shielded membrane lipids from peroxidation. In comparison to a placebo, clinical research produced statistically significant increases in dermatologist scores and subject self-assessments for skin texture, hydration, tone, firmness, and radiance. These investigations substantiate the application of this standardised TC fruit extract as a preventative measure to assist shield skin from long-term exposure to environmental pollutants, as well as a restorative to lessen obvious indications of pre-existing damage.

Singh R, Kumar S, et al. (2021) abstracted that the goal of the current study was to evaluate the in vitro antioxidant activities and their relationship to the phenolic and flavonoid content found in fruit extracts from *Emblica officinalis* and *Terminalia chebula*, which are commonly used in Indian herbal cuisine. Radicals like DPPH were used to evaluate the extract's capacity to scavenge radicals. 70% fruit extracts from both the plant were used for the examination of antioxidant activity. As a result, it shows that the antioxidant quality of *Terminalia chebula* is greater than *Emblica officinalis*. The ascorbic acid, phenolic and flavonoid standards relates that the antioxidant properties were examined. As a conclusion, according to DDPH radical experiment, , the flavonoid and phenolic content were measured as follows: 127.60 ± 0.001 mg/ml for flavonoid content and 133.00 ± 0.003 mg/ml for phenolic content expressed as gallic acid equivalent per 100 mg of fruit extract respectively.

Choudhary RA, Manivannan E, Chandrashekar R, et al. (2021) determined that *Terminalia chebula* was most widely used in traditional system of medicine. The goal of the current study is to test EEFTC fruit pulp for

phytochemicals and determine its medicinal properties. They conducted a experiment on dried fruit of *Terminalia chebula* by using screening techniques such as smoothie preparation, phytochemical analysis, evaluation of the determination of crude fibre content and antimicrobial activity of plant extracts, and qualitative phytochemical analysis. As a result, they observed that that the aqueous, ethanolic extract of *T. Chebula* (EETC) is rich with all the compound like phytosterols, triterpenoids, carbohydrates, glycosides, and phenolic compound. At various concentrations the dried pulp extract of *Terminalia chebula* was examined for its antimicrobial activity. If the antimicrobial activity was greater than 8 mm, it was indicated by an inhibitory zone encircling the extract well. Biomolecules found in *Terminalia chebula* have demonstrated antiviral, anti-inflammatory, and antioxidant effects against a variety of pathogen microorganisms. In the current pandemic epidemic scenario, a prompt screening of therapeutically bioactive ingredients is necessary to confirm these herbs anti-SARS-CoV-2 ability.

Nigam M, Mishra AP, Adhikari-Devkota A, et al. (2020) reported that fruits of *Terminalia chebula* Retz are used as crude medications. The major desire of this article is to evaluate the available data of *Terminalia Chebula*'s traditional usage, bioactive chemical components and pharmacological activity. They determined that many phytochemicals including tannins, phenolic acids, flavonoids and other beneficial substances are present in *Terminalia Chebula* according to many studies. *Terminalia chebula* has also examined to have many pharmacological properties including its cytotoxic, hepatoprotective, neuroprotective, antioxidant and anti-inflammatory properties. Future research should be carried out to strengthen the evidence supporting their traditional applications, more in vivo and clinical research for mechanism -based pharmacological evaluation.

Sabir SM, Abbas SR, Shahida S, et al. (2020) examined the determination of bioactive compounds and the antioxidant activity of extracts of *Terminalia chebula*. The DPPH assay, lipid peroxidation assay, iron chelation assay, and total antioxidant assay were used to measure the antioxidant activity. HPLC-DAD was used to determine the phenolic content. Using high performance liquid chromatography (HPLC) to identify the specific phenolic contents, antioxidant substances such as



quercetin, ellagic acid, rutin, catechin, caffeic acid, gallic acid, and epicatechin were found. AdmetSar was used to verify the ADMET properties. In the phospholipid homogenate of egg yolks, the aqueous extract demonstrated the ability to reduce lipid peroxidation. It is established that *T. chebula* extracts are rich in phenolics and have strong anti-inflammatory and antioxidant properties.

Shendge AK, Sarkar R, Mandal N, et al. (2020) examined the anti-inflammatory and anticancer properties of *Terminalia chebula* fruit (TCME). Using A549 and MCF-7 cells, the TCME's in vitro anticancer efficacy was assessed. A549 and MCF-7 cells were both susceptible to cytotoxicity from TCME. Following TCME treatment, confocal microscopy revealed DNA fragmentation in both cell lines. Furthermore, in both cell lines, TCME administration causes the activation of caspase-cascade pathways linked to apoptosis. Ultimately, the bioactive phytochemicals in TCME were identified by HPLC analysis. The combined data demonstrated the fruit of *Terminalia chebula*'s strong anti-inflammatory and anticancer effects.

Nigam M, Mishra AP, Adhikari-Devkota A, et al. (2020) studied that the most commonly used crude drug in various traditional medicine system is *Terminalia chebula* which belongs to the family *Combretaceae*. They review the available data of *Terminalia chebula* traditional uses, bioactive chemical constituents and the pharmacological activities. According to many researchers, *Terminalia chebula* consists of many phytochemicals such as flavonoids, tannins, phenolic acids and other bioactive compounds. They also studied regarding their pharmacological activities such as antioxidant, hepatoprotective, neuroprotective, cytotoxic, antidiabetic, anti-inflammatory activities. However, more in vivo and clinical studies for mechanism-based pharmacological evaluation should be conducted in future to provide stronger scientific evidences for their traditional uses.

Rafik U Shaikh et al., (2015) researched that evaluation of in vivo and in vitro anti-inflammatory potential of medicinal plants utilized in traditional medicines. The extraction process of *Terminalia chebula* was carried out by water, ethanol and hexane. It was examined by invitro for COX-1 and 2 inhibitory and antioxidant effect. The in vivo anti-inflammatory activity was processed using

carrageenan and phorbol Myristate Acetate(PMA). As the result, compared to COX-1 most of the plants inhibit the COX-2 activity. Effective DPPH, OH and superoxide radical scavenging activity were showed in the ethanol extract of the sample. *Terminalia chebula* had an impact on inhibition of edema formation which is examined by in vivo anti-inflammatory study. The cytotoxicity evaluation study states that there is no effect on cell viability. This study maybe beneficial in strengthening the standardization of the sample. It can also be considered as a resource for anti-inflammatory agents possessing COX-2 inhibition.

- The selected medicinal herb *Terminalia chebula* is extracted using ethanol by the process of soxhlet extraction method.
- The residue is used to formulate the herbal ointment.
- The ointment is evaluated for its parameters.
- It is tested for its anti-oxidant activity by using DPPH assay.
- The anti-bacterial activity of the ointment is determined by using Muller-Hinton Agar.

2. METHODS AND MATERIALS:

3.1 Selection of plant

Terminalia chebula is a medicinal plant and its fruit is called as Haritaki, belongs to the family *Combretaceae*, which is used in ayurvedic medicine. It grows throughout India, Burma and Sri Lanka. It has various chemical constituents such as chebulagic acid, chebulinic acid, gallic acid etc., It is very essential which depends on the requirement to isolate an active compound. Selection of active compounds is based on environment against insects and bacteria.

3.2 Experimental methodology

Identification of plant: The species is identified by dark brown bark exfoliating in irregular woody scales and by the presence of a pair of large glands at the top of the petiole. It is a medium to large deciduous tree growing to 30m tall, with a trunk up to 1m in diameter. Myrobalans are the dried fruits of *Terminalia chebula*. The immature fruits are black, ovoid and about 1-3 cm long.

Collection of plant: Fruits are collected in summer in May- June when they turn yellow. The seeds can be collected as soon as they fall on the ground and are dried



under shade. It can be stored in gunny bags for one year, but fresh seeds germinate quicker.

Drying and Grinding of the plant: After cleaning of the collected plants, they were cut into small pieces by using knives and scissors. The collected plants are well dried in shade for a few days, with moisture content not more than 10%, and stored in well-ventilated containers to protect from contamination and dust present in the environment. After drying completely, it is grinded to obtain fine powder to enhance the surface area for better extraction process.

3.3 FORMULATION:

Ointments are viscous, semisolid preparations containing either dissolved or suspended functional ingredients. It is used on the skin to soothe or heal wounds, burns, rashes, scrapes or other skin problems. It has a healing property. It is applied topically to heal the skin. Ointment base is used to provide effective penetration and absorption in the layers of stratum corneum.

The types of ointment base are,

- Hydrocarbon bases
- Absorption bases
- Water removable bases
- Water soluble bases

S no	INGREDIENTS	FORMULA (100 g)
1	White Bees Wax	16 g
2	Liquid Paraffin	50 ml
3	Borax	0.8 g
4	Purified water	33 ml
5	Rose oil	0.1 ml
6	Methyl Paraben	0.18 g
7	Terminalia Chebula extract	2 g

White bees wax is used as soothing agent in herbal ointment. Liquid paraffin is used as moisturizer and protective agent in herbal ointment. Borax is used to remove dead cells and excess oil. Purified water is used as the base for the herbal ointment. Rose oil is used as

flavoring agent in herbal ointment. Methyl paraben is used as preservative in herbal ointment. Terminalia chebula extract used as skin layer thickening.

3.4 PROCEDURE:

1) Dissolve borax in hot rose water. Also add alcoholic extract of herbal crude drug, if any used in the formulation.

2) Melt various waxes together keeping the temperature about 70°C. Ingredients of oil phase should be taken in increasing melting point. The materials of least melting point should be taken and melt it. Add the other oil or wax gradually in increasing melting point and melt them with continuous stirring.

3) Take separately the ingredients of aqueous phase and mix them and heat to same temperature as oil phase with constant stirring.

4) Mix without heat for 1hr and when cool (45-50°C), add perfume, for eg. red apple extract or other perfume. Preservative should be dissolved in the water before making ointment, if required. Perfume should be added after the primary ointment is formed and cooled but before final milling.

3.5 EVALUATION:

Evaluation test is defined as the method of analysis such as colour, odor, special features, texture, etc. Evaluation test determines the quality of the product. A perfect product can be prepared and marketed after evaluation test.

Various type of evaluation test is

- pH measurement
- Appearance
- Viscosity
- Saponification value
- Acid value
- Irritancy test
- Accelerated stability testing
- Homogeneity
- Microbial examination of ointment
- Determination of spreadability
- Determination of total fatty acid
- Determination of water content



1) pH MEASUREMENT:

The pH meter was calibrated using standard buffer solution. About 0.5 gm of the ointment was weighed and dissolved in 50ml of distilled water and its pH was measured using digital pH meter.

2) APPEARANCE:

The appearance of the ointment was judged by its colour, pearlescence, roughness and graded.

3) VISCOSITY:

It was determined by Brookfield viscometer at 100rpm, using spindle no. 7 at temperature of 25°C. The determinations were carried out in triplicate and the average of three readings was recorded.

4) ACID VALUE:

Take 10gm of the substance dissolved in accurately weighed in 50ml mixture of equal volume of alcohol and solvent ether. The flask was connected to reflux condenser and slowly heated. Until sample was dissolved completely. To this, 1ml of phenolphthalein added and titrated with 0.1N NaOH, until faintly pink colour appears after shaking for 30 seconds.

$$\text{Acid Value} = n \times 5.61/W$$

5) SAPONIFICATION VALUE:

Introduce about 2gm of the substance refluxed with 25ml of 0.5N alcoholic KOH for 30minutes. Add 1ml of phenolphthalein and titrate immediately with 0.5N HCL.

$$\text{Saponification Value} = (b-a) \times 28.05/W$$

W

6) IRRITANCY TEST:

7) Mark an area (1 sq. cm) on the left-hand dorsal surface. The ointment was applied to the specified area and time was noted. Irritancy, erythema, edema was checked if any for regular intervals upto 24hours and reported. As there was no reaction the test was repeated three times. As no reaction was observed on third application, the person may be taken as not hypersensitive.

8) ACCELERATED STABILITY TESTING:

It was conducted for 2 most stable formulations at room temperature, studied for 7days. The formulation was placed at 40°C ± 1°C for 20days. Both formulations were

kept at room and elevated temperature and observed on 0th, 5th, 10th, 15th and 20th day for any change in colour, phase separation, etc.

9) HOMOGENICITY:

The formulations were tested for homogeneity by visual appearance and by touch.

10) DETERMINATION OF PREADABILITY:

Spreadability may be expressed by extent of the area to which the topical application spreads when applied to the affected parts on the skin. The therapeutic efficiency of the formulations also depends on its spreading value. Sample (about 2gm) was applied in between two glass slides and they were pressed together to obtain a film of uniform thickness by placing 1000gm weight for 5 minutes. Thereafter a weight (10gm) was added to the pan and the top plate was subjected to pull with the help of string attached to the hook. The time in which the upper glass slide moves over the lower plate to cover a distance of 10cm is noted. The spreadability is measured by using the formula:

$$S = m \times L/T$$

M = weight tied to upper glass slide

L = Length on a glass slide

T = Time taken

11) MICROBIAL EXAMINATION OF OINTMENT:

The formulated ointment was inoculated on plates of agar media by streak plate method and a control was prepared by omitting the ointment. The plates were placed into the incubator and are incubated at 37°C for 24hrs. After the incubation period, plates were taken out and check the microbial growth by comparing it with control.

12) DETERMINATION OF TOTAL FATTY MATTER:

2g of the sample was weighed in a conical flask, added with 25ml of dil. HCL (1%v/v) & refluxed. Poured this into the separating funnel and 50ml of ethyl ether were added in to it. The separating funnel was shaken well until two layers were separated. The aqueous layer was separated out and added 50ml portion of ether twice. All the ether extracts were combined and filter through the



1,1-Diphenyl-2-picrylhydrazil (DPPH)



temperature for 30 min. The absorbance of the solution at 517 nm was recorded. A mixed solution with 1,200 µl of ethanol and 800 µl of Tris-HCL buffer (Ph 7.4) was used as the blank.

The inhibition ratio (%) was obtained from the following equation:

Chemicals required:

SNO	CHEMICALS	MICROLITER [µL]	MILLILITER [ml]
1	DPPH	1000 µl	1 ml
2	Tris-HCL buffer	800 µl	0.8 ml
3	Ethanol	1,200 µl	1.2ml
4	Sample solution	200 µl	0.2ml

Evaluation:

The serial dilution was performed for the test sample at the concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5. The

$$\text{Inhibition ratio (\%)} = [(A1 - A2) / A1] \times 100,$$

Where A1 is the absorbance of the addition of ethanol instead of testing sample and A2 is the absorbance of testing sample solution.

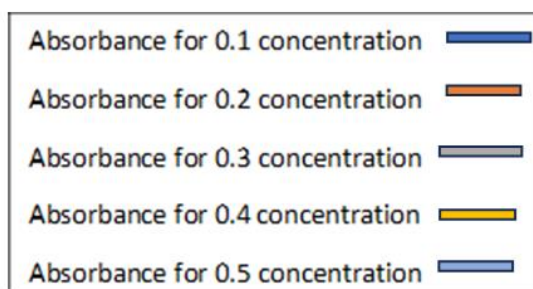
solutions at different concentrations are evaluated for its absorbance by using UV- Spectroscopy. The absorbance is obtained for the test samples at a range of 400-800nm. The observed data are,

WAVELENGTH	0.1 CONC ABSORBANCE	0.2 CONC ABSORBANCE	0.3 CONC ABSORBANCE	0.4 CONC ABSORBANCE	0.5 CONC ABSORBANCE
515	0.073	0.09	0.11	0.118	0.204
530	0.064	0.076	0.093	0.099	0.173
545	0.057	0.065	0.078	0.084	0.148
560	0.05	0.055	0.067	0.072	0.126
575	0.045	0.048	0.057	0.061	0.108
590	0.041	0.042	0.049	0.053	0.093
605	0.037	0.037	0.043	0.046	0.082
620	0.034	0.032	0.037	0.04	0.071
635	0.031	0.028	0.033	0.035	0.063
650	0.024	0.02	0.023	0.025	0.051
665	0.025	0.02	0.023	0.024	0.048



680	0.024	0.018	0.021	0.022	0.038
695	0.022	0.015	0.018	0.019	0.038
710	0.02	0.013	0.015	0.016	0.033
725	0.02	0.012	0.014	0.014	0.03
740	0.018	0.01	0.012	0.012	0.026
755	0.018	0.009	0.011	0.01	0.023
770	0.014	0.005	0.006	0.005	0.016

The pictorial representation for the absorbance of different concentrations at 400-800nm range,

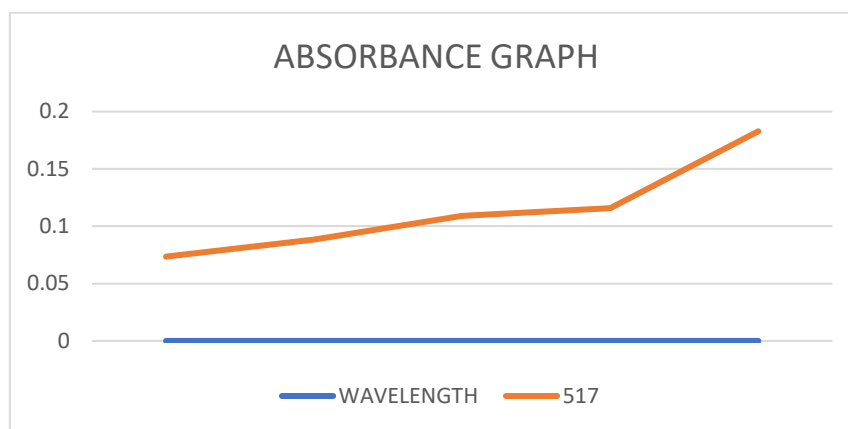


The graph represents that highest value for λ_{max} was observed at 517nm for all the concentrations. The absorbance value for the blank is determined as 0.52 at

517nm. The absorbance value for all the concentrations at 517nm are,

TEST SAMPLE	WAVELENGTH (nm)	ABSORBANCE
Sample 1	517	0.0735
Sample 2	517	0.0885
Sample 3	517	0.1091
Sample 4	517	0.1158
Sample 5	517	0.1828

The pictorial representation for the absorbance obtained at λ_{max} 517nm for the test sample,



The graph indicates that absorbance increases as the concentration of the test solution increases. The % scavenging for all the concentration's is calculated to

determine which concentration has the highest antioxidant activity

$$\text{Inhibition ratio (\%)} = [(A1 - A2) / A1] \times 100$$

CONCENTRATIONS	CONTROL	TEST SAMPLE	% INHIBITION RATIO
0.1	0.52	0.0735	85.86
0.2	0.52	0.885	82.98
0.3	0.52	0.1091	79.01
0.4	0.52	0.1158	77.73
0.5	0.52	0.1828	64.84

The 0.1 concentration has the highest inhibition ratio compared to other concentrations. Thus, 0.1 concentration has the highest antioxidant activity.

6.1.4 DPPH SCAVENGING FOR ASCORBIC ACID:

The DPPH assay is carried out with ascorbic acid as the standard drug. The mixture of ethanol and Tris-HCl solution is used as the blank. The mixture of DPPH solution, Tris-HCl buffer and Ascorbic acid solution is kept at dark for 30mins at room temperature. The testing

solution with ascorbic acid is performed a serial dilution process. Thus, the dilution is done at the concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5.

EVALUATION:

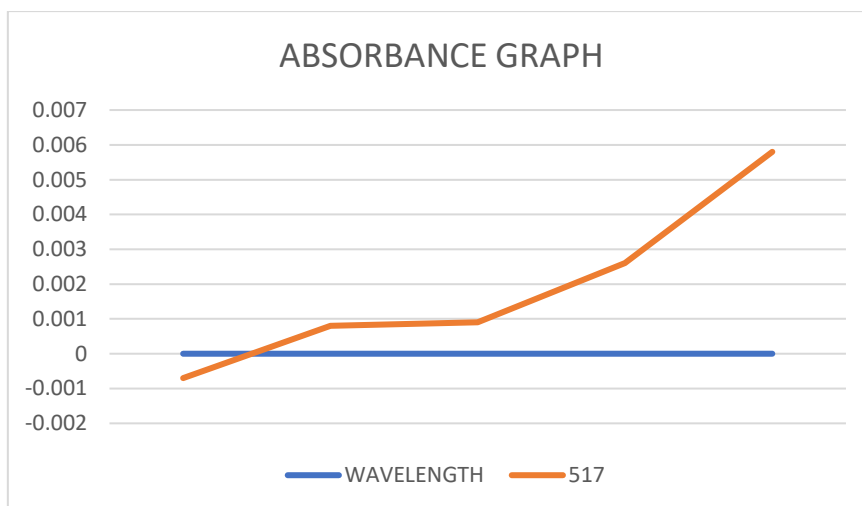
The absorbance is determined by using UV-Spectroscopy. The absorbance value for the blank is found as 0.0299 at 517nm. The absorbance for the various concentrations at λ_{max} 517nm is,

TEST SAMPLE	WAVELENGTH (nm)	ABSORBANCE
Sample 1	517	-0.0007



Sample 2	517	0.0008
Sample 3	517	0.0009
Sample 4	517	0.0026
Sample 5	517	0.0058

The pictorial representation for the absorbance obtained at λ_{max} 517nm for ascorbic acid,



The graph shows that absorbance increases as the concentration of the test solution increases. The % scavenging for all the concentration's is calculated to determine which concentration has the highest antioxidant activity.

$$\text{Inhibition ratio (\%)} = [(A1 - A2) / A1] \times 100$$

CONCENTRATIONS	CONTROL	SAMPLE	% INHIBITION RATIO
0.1	0.0299	-0.0007	-
0.2	0.0299	0.0008	97.32
0.3	0.0299	0.0009	96.98
0.4	0.0299	0.0026	91.30
0.5	0.0299	0.0058	80.6

As the absorbance value for 0.1 concentration is negative it is neglectable. So, 0.2 concentration has the highest inhibition ratio. Thus, 0.2 concentration shows more

antioxidant activity than compared to other concentrations.



3.1 ANTIBACTERIAL ACTIVITY

The antibacterial activity is determined by the microbial test. This activity is evaluated to determine its effect against bacteria or micro-organisms.

3.1.1 PREPARATION OF EXTRACTS SOLUTION

The dried extracts were dissolved to make a solution in Dimethyl sulfoxide (DMSO). For uniform mixing, the solution was kept in centrifuge for 25 mins at 13000rpm. Ciprofloxacin(50mg/ml) is a standard marketed antibiotic which is used for comparing the activity with extraction solution.

Microbes used in the test: The microorganism such as *Escherichia coli*, *salmonella* and *pseudomonas* species were used for this study. The microbes used for the activity, which belongs to Gram negative bacteria.

3.1.2 CULTURE MEDIA PREPARATION

To test the antibacterial activity, MHA (Muller-Hinton Agar) is used for the media preparation. The culture media were prepared in 250ml distilled water by dissolving 9.5g of MHA. The obtained solution is mixed thoroughly and boiled with frequent agitation to dissolve agar powder completely. Then, autoclave the media for sterilization at 15 lbs pressure, at 121°C temperature, for 15 minutes. Allow the media to cool down in laminar flow hood. The 25ml of the media were poured into each petri plate and leave for few minutes to all it to solidify. After solidification, spread the culture microbes on media by using cotton swab and cover the whole media with turn 90° rotation without leaving any gap. Make 6 holes in each petri plate. The first 4 holes are filled with 30 µl of each fraction and the antibiotics in the second last holes and solvent in the last hole. For sterility of media negative control, one petri plate is placed without any microbes, whereas for positive control, two plates are placed for both microbes with no antibiotics and extraction fraction added. Finally, the petri plates are stored for incubation in bio-chemical oxygen demand (BOD) incubator at 37°C for 24 hours.

4. RESULTS AND DISCUSSION

The formulated herbal ointment using *Terminalia chebula* has been evaluated for its parameters. The parameters evaluated are its appearance, irritancy test, spreadability, stability test, water content, pH,

Homogeneity, viscosity, acid value, saponification, microbial test and total fatty matter.

The evaluated parameters resulted that the ointment has the colour and standard in its appearance. The ointment passes the irritancy and spreadability test. The ointment is stored in room temperature for a week and resulted that it is stable. The pH of the ointment is evaluated as acidic as the value for pH is 4.0. The Homogeneity test is carried out and determine as positive. The viscosity of the product is determined to measure the flow rate of the product and it is resulted as . The acid value is evaluated as 5.61 and it resulted that it is acidic in nature. The saponification value is determined as 14 which reported that it hydrolyses fats. The microbial test is carried out with three different micro-organisms such as *Escherichia coli*, *Salmonella* and *Pseudomonas*.

Oxidative stress is prevented by the antioxidant present in the medicinal herb. The medicinal herb *Terminalia chebula* showed antioxidant activity. The test sample (*Terminalia chebula*) showed highest effect of antioxidant activity at 0.1 concentration. The % scavenging (Inhibition ratio) of the test sample at 0.1 concentration is determined as 85.86 %. The standard (Ascorbic acid) showed highest effect of antioxidant activity at 0.2 concentration. The % scavenging (Inhibition ratio) of the standard sample at 0.2 concentration is determined as 97.32 %. Thus, the result indicates that the extract of *Terminalia chebula* has the highest antioxidant activity than that of Ascorbic acid. The free radical scavenging activity of ethanolic extract was confirmed in the present investigation.

However, the chemical constituents like tannins, reducing sugar and proteins are essential for the antioxidant activity of the selected medicinal herb. The phytochemical test performed and confirmed the presence of alkaloids, flavonoids, steroids, tannins and glycosides in the extract. Thus, the medicinal herb has highest antioxidant activity.

The antibacterial effect of the ethanolic extract of *Terminalia chebula* on *Escherichia coli* would depend on various factors such as the concentration of the extract, the method of extraction and the susceptibility of the specific strain of *Escherichia coli*, *pseudomonas* and *salmonella* being tested. It showed highest activity against *Escherichia coli* (15.33±0.67) and lowest activity against *Salmonella* (12.00±0.58) for standard drug. The



ethanolic extract of *Terminalia chebula* shows highest activity against *Escherichia coli* (12.00 ± 0.58) and lowest activity against *Salmonella* (9.00 ± 0.58) respectively.



Materials	Name of organism	Zone of inhibition(mm)
Ciprofloxacin as a standard	<i>E.coli</i>	15.33 ± 0.67
	<i>Pseudomonas</i>	13.33 ± 0.67
	<i>Salmonella</i>	12.00 ± 0.58

Materials	Name of organism	Zone of inhibition(mm)
Ethanol extract of <i>Terminalia chebula</i> (EETC)	<i>E.coli</i>	16.00 ± 0.58
	<i>Pseudomonas</i>	14.27 ± 0.82
	<i>Salmonella</i>	12.98 ± 0.58

5. CONCLUSION:

The study was carried out to evaluate the antibacterial and antioxidant activity by using DPPH assay. The tested ethanolic extracts of *Terminalia chebula* has a significant antioxidant effect that has been determined. It was found that ethanolic extracts of *Terminalia chebula* has very excellent antibacterial activity and pathogenic bacteria were inhibited. The study proved that the ethanolic extracts of *Terminalia chebula* have antibacterial agents against various human pathogenic bacteria. This study provides invitro anti-oxidant and anti-bacterial studies to

understand the action of *Terminalia chebula* which may help in developing better therapeutic agents.

The selected medicinal herb *Terminalia chebula* is extracted and formulated as ointment. The phytochemical constituents present in the sample are examined. The ointment is evaluated and it is examined for its antioxidant and antibacterial activity.

It results that the herb *Terminalia chebula* has many properties and it is more effective. It reported that it has antioxidant and antibacterial activity. We conclude that *Terminalia chebula*, the medicinal herb is used as an antibiotic ointment and has the power of healing.



REFERENCE:

1. Anwesa Bag, Subir Kumar Bhattacharyya, Nishith kumar pal rabi ranjan Chattopadhyay. Anti-inflammatory, anti-lipid peroxidative, antioxidant and membrane stabilizing activities of hydroalcoholic extract of Terminalia chebula fruits. pharmaceutical biology, 2013, 51.
2. E. Soruba, Vickram Sundaram, R. P. Manian, Karikalan Kulanthaivelu, Sridharan Balasundaram. Antioxidant, antibacterial and anti-inflammatory activity of *Acalypha indica* and *Terminalia chebula*: an *in-vitro* analysis. Research journal of pharmaceutical, Biological and chemical sciences, 2015, 6(3), 180-188.
3. Min hye Yang, Zulfiqar ali, ikhlas A. khan, shabana I. khan. Anti-inflammatory activity of constituents isolated from Terminalia chebula. sage journals, 2014.
4. Hua yew chwnq, Ta chen lin, kuo hua yu, chien min yang, chun ching lin. Antioxidant and free radical scavenging activities of Terminalia chebula. biological and pharmaceutical bulletin, 2003, 26(9), 1331-1335.
5. Ekambaram sanmuga priya, Perumal senthamil selvan, Balakrishnan ajay. Tannin rich fraction from Terminalia chebula fruits as anti-inflammatory agent. Journals of herbs, spices and medicinal plants, 2018, 24.
6. Fan Xiao, tao xu, baiyi lu, ruihai liu. Guideliness for antioxidant assays for food components. food frontiers, 2020.
7. G. Leelaprakash, S. Mohan Dass. In vitro anti-inflammatory activity of methanol extract of *enicostemma axillare*. International journal of drug development and research, 2011, 3.
8. Syed mubashar sabir, Syed rizwan abbas, Shabnam shahida, Muhammad faraz khan. Invitro antioxidant, anti-lipid peroxidative activities and in silico study of Terminalia chebula bioactive compounds. clinical Phytoscience, 2020, 6.
9. Varma K, Vishwakarma G, Pathak R. Extraction phytochemical screening and development of phytosomes of hydroalcoholic extract of Terminalia chebula Retz for hepato-protection. Journal of Drug Delivery and Therapeutics, 2019, 9(3), 397-406.
10. Akhtar N, Khan AB, Muhammad S, Ahmed M, Khan HM, Rasool F, Saeed T. Formulation and characterization of a cream containing terminalia chebula extract. Forschende Komplementärmedizin/Research in Complementary Medicine, 2012, 19(1), 20-25.
11. Vidya sabale, Harish kunjwani, Prafulla sabale. Formulation and in vitro evaluation of the tropical antiageing preparation of the fruit of Benincasa hispida. Journal of Ayurveda and integrative medicine, 2011, 124-128.
12. Sarmistha saha, Ramtej j. verma. Anti-oxidant activity of polyphenolic extracts of Terminalia chebula retzius fruits. Journal of Taibah university for science, 2016, 10, 805-812.
13. Gulcin İ, Alwasel SH. DPPH radical scavenging assay. Processes, 2023, 11(8), 2248.
14. Shah P, Modi HA. Comparative study of DPPH, ABTS and FRAP assays for determination of antioxidant activity. International Journal for Research in Applied Science and Engineering Technology, 2015, 3(6), 636-41.
15. Mishra K, Ojha H, Chaudhury NK. Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. Food chemistry, 2012, 130(4), 1036-1043.
16. Deng J, Cheng W, Yang G. A novel antioxidant activity index (AAU) for natural products using the DPPH assay. Food Chemistry, 2011, 125(4), 1430-1435.
17. Amir hassan, Himayat ullah. Antibacterial and antifungal activities of the medicinal plant veronica biloba. Journal of chemistry, 2019.
18. Ghosh A, Das BK, Roy A, Mandal B, Chandra G. Antibacterial activity of some medicinal plant extracts. Journal of natural medicines, 2008, 259-262.
19. Nascimento GG, Locatelli J, Freitas PC, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Brazilian journal of microbiology, 2000, 247-256.
20. Tan JB, Lim YY. Critical analysis of current methods for assessing the in vitro antioxidant and antibacterial activity of plant extracts. Food chemistry, 2015, 814-822.
21. Bhujbal A, Mohite M, Alhat S, Adhe S, Kure N, Khedkar S. In Vitro Evaluation of Polyherbal Cream as a Promising Therapeutic Approach for Psoriasis:



- Assessing Anti-inflammatory and Anti-Fungal activity. *Lampyrid: The Journal of Bioluminescent Beetle Research*, 2023, 13, 651-666.
22. Choudhary RA, Manivannan E, Chandrashekar R, Ravi I, Sivasankari V, Arul AK. Phytochemical analysis of ethanolic extract of fruits of *Terminalia chebula* and its medicinal use in human. *Phytochemical analysis*, 2021, 2, 43-54.
 23. Guleria S, Kumar V, Chandel SR, Sharma N, Sourirajan A, Khosla PK, Dev K. Antioxidant and antimicrobial activity of ethanolic extract and its fractions from fruit and leaves of *terminalia chebula* from Himachal Pradesh, India. *Plant Archives*, 2020, 2, 4753-4761.
 24. Kumar S, Chauhan N, Tyagi B, Yadav P, Samanta AK, Tyagi AK. Exploring bioactive compounds and antioxidant properties of twenty-six Indian medicinal plant extracts: A correlative analysis for potential therapeutic insights. *Food and Humanity*, 2023, 1, 1670-1679.
 25. Kim HJ, Song HK, Park SH, Jang S, Park KS, Song KH, Lee SK, Kim T. *Terminalia chebula* Retz. extract ameliorates the symptoms of atopic dermatitis by regulating anti-inflammatory factors in vivo and suppressing STAT1/3 and NF- κ B signaling in vitro. *Phytomedicine*, 2022, 104.
 26. Nigam M, Mishra AP, Adhikari-Devkota A, Dirar AI, Hassan MM, Adhikari A, Belwal T, Devkota HP. Fruits of *Terminalia chebula* Retz.: A review on traditional uses, bioactive chemical constituents and pharmacological activities. *Phytotherapy Research*, 2020, 10, 2518-2533.
 27. Na Takuathung M, Wongnoppavich A, Jaijoy K, Soonthornchareonnon N, Sireeratawong S. Antioxidant and Antitumorigenic Activities of the Standardized Water Extract from Fruit of *Terminalia chebula* Retz. var. *chebula*. *Natural Product Communications*, 2023, 6.
 28. Randhawa M, Meyer T, Sachdev M, Chaudhuri RK. Standardized *terminalia chebula* fruit extract: a natural ingredient that provides long-lasting antioxidant protection and reverses visible signs of pollution-induced skin damage. *Clinical, Cosmetic and Investigational Dermatology*, 2021, 1257-1269.
 29. Singh AK, Kumar P, Rajput VD, Mishra SK, Tiwari KN, Singh AK, Minkina T, Pandey AK. Phytochemicals, Antioxidant, Anti-inflammatory Studies, and Identification of Bioactive Compounds Using GC-MS of Ethanolic Novel Polyherbal Extract. *Applied Biochemistry Biotechnology*, 2023, 7, 4447-4468.
 30. Shendge AK, Sarkar R, Mandal N. Potent anti-inflammatory *Terminalia chebula* fruit showed in vitro anticancer activity on lung and breast carcinoma cells through the regulation of Bax/Bcl-2 and caspase-cascade pathways. *Journal of Food Biochemistry*, 2020, 12.
 31. Sultan MT, Anwar MJ, Imran M, Khalil I, Saeed F, Neelum S, Alsagaby SA, Al Abdulmonem W, Abdelgawad MA, Hussain M, El-Ghorab AH. Phytochemical profile and pro-healthy properties of *Terminalia chebula*: A comprehensive review. *International Journal of Food Properties*, 2023, 1, 526-551.
 32. Singh R, Kumar S. Study on correlation of Antioxidant activities with presence of phenolic and Flavanoid contents in *Emblica officinalis* and *Terminalia chebula*. *Journal of Drug Delivery and Therapeutics*, 2021, 1, 32-35.
 33. Shaikh, R. U., Pund, M. M., & Gacche, R. N. Evaluation of anti-inflammatory activity of selected medicinal plants used in Indian traditional medication system in vitro as well as in vivo. *Journal of traditional and complementary medicine*, 2015, 4, 355–361.
 34. Thoithoisana Devi S, Devika Chanu K, Singh NB, Chaudhary SK, Keithellakpam OS, Singh KB, Mukherjee PK, Sharma N. Chemical Profiling and Therapeutic Evaluation of Standardized Hydroalcoholic Extracts of *Terminalia chebula* Fruits Collected from Different Locations in Manipur against Colorectal Cancer, *Molecules*. 2023, 7.
 35. Suchalatha S, Devi CS. Antioxidant activity of ethanolic extract of *Terminalia chebula* fruit against isoproterenol-induced oxidative stress in rats, 2005, 42, 246-249.
 36. Chang CL, Lin CS. Phytochemical composition, antioxidant activity, and neuroprotective effect of *Terminalia chebula* Retzius extracts. *Evidence-Based Complementary and Alternative Medicine*, 2012.
 37. Chaves N, Santiago A, Alias JC. Quantification of the antioxidant activity of plant extracts: Analysis of sensitivity and hierarchization based on the method used. *Antioxidants*, 2020, 1, 76



-
38. Lee, J., Nho, Y.H., Yun, S.K. Use of ethanol extracts of *Terminalia chebula* to prevent periodontal disease induced by dental plaque bacteria, 2017, 17
 39. Prusti.A, Mishra.S. R, Sahoo.S, Mishra, S.K. Antibacterial activity of some Indian medicinal plants. ethnobotanical leaflets, 2008, 12, 227-230.
 40. BE Ravi Shankara, YL Ramachandra, S Sundara Rajan, J Preetham, PS. Sujan Ganapathy. In vitro antibacterial activity of *Terminalia chebula* leaf gall extracts against some human pathogenic strains. International current pharmaceutical journal, 2012, 1, 217-220.