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Research

Extraction, Preliminary Phytochemical Screening & Antibacterial Activity of *Terminalia chebula* Fruit

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Abstract

The aim of the current research investigation was to perform the extraction, preliminary phytochemical screening and to evaluate the *invitro* antibacterial activity of fruit of *Terminalia chebula*. The present study includes the extraction with methanol and screening of various active chemical constituents' of fruit of *Terminalia chebula* that exhibit anti-microbial activity. The fruit of *Terminalia chebula* was screened for estimation of phytochemical standards like: phenolic, flavonoid, tannins, carbohydrates, steroids, glycosides and mucilage content. In the current investigation, Methanol extractions are subjected to *invitro* antibacterial activity using standard procedures. The investigation was accomplished using agar well diffusion method. Outcomes of Phytochemical analysis of *Terminalia chebula* revealed that the plant possesses strong antibacterial potentials. The zone of inhibition for methanol extract against Staphylococcus aureus at 100µL was found to be 4.8 mm. The zone of inhibition for methanol extract at 100µL against E.coli was found to be 3.0 mm and The zone of inhibition for standard drug ampicillin was found to be 6.2mm.The existence of the bioactive compounds in the fruit may be responsible for antibacterial properties of *Terminalia chebula* that form the basis of their use in herbal medicine in India. The evidence found from contemporary study deliver valuable data that will be supportive in identifying and to perform upcoming research in future.

Keywords: *Terminalia chebula,* Methanol, zone of inhibition, *Invitro* antibacterial activity

INTRODUCTION

Humans have always been fascinated by illness and mortality. From the very beginning, theories have circulated that illness could be brought on by the invasion of the body by outside agents that are inhaled or consumed. Microorganisms are of incalculable value to Earth's ecology, disintegrating animal and plant remains

and converting them to simpler substances that can be recycled in other organisms. *Terminalia chebula* is known to have various medicinal properties. *Terminalia chebula* Retzius (*T. chebula* Retz) is a medium to large-sized tree that belongs to the Combretaceae family and is widely distributed throughout Asia. Ayurvedic remedies are widely used to treat human health complications like digestive, tonic, antipyretic, spasmolytic, astringent, expectorant, antiasthmatic, antiviral, and hypoglycemic conditions. Plants containing polyphenols have been reported to possess strong antibacterial properties. The antimicrobial susceptibility was screened using the disc diffusion method and the minimum inhibitory concentration (MIC) was determined using the broth microdilution method. The results showed that it was active against both gram-positive and gram-negative bacteria. Ethanol extract of *Terminalia chebula* was studied for its antibacterial activity against clinically important standard reference bacterial strains The results showed that it was active against both gram-positive and gram-negative bacteria. The *T. chebula* fruit extract was highly effective against *Salmonella typhi*.[1] *Terminalia chebula* belonging to the family Combretaceae is called the "King of Medicines" in Tibet and used in the treatment of various diseases like diabetes, depression, memory loss, cardiovascular diseases, leprosy etc and it also inhibits the growth of malignant tumors. *T.chebula* fruit is rich in phytochemical constituents such as tannins, flavonoids and essential oils.[2] In the above backdrop, an attempt was made in this current research to screen *Terminalia chebula* for various phytoconstituents and by subjecting it to antibacterial activity.



Fig 1: Fruits of *Terminalia chebula*

MATERIALS AND METHODS

Collection and authentication of plant

Terminalia Chebula (Combretaceae) fruits were collected from Chittoor district Andhra Pradesh, India in march 2024. The selected material was identified by Dr. K. Madhava Chetty, M.s.c.,M.Ed.,M.Phil., PGD., Ph.D., Plant Taxonomist (IAAT: 357), Assistant Professor, Botany Department. Sri Venkateshwara University, Tirupati. Later, a voucher sample of the same was placed in herbarium for reference (1804).



Fig 2: Whole plant of *Terminalia chebula*

Extraction

Terminalia chebula fruits were dried out in shade and crushed to obtain a coarse powder. [3,4] The material was passed over a sieve and kept in a plate. Material of *Terminalia chebula* subjected to continuous Soxhlet extraction method to extract with methanol. [5] By applying rotary vacuum evaporator, the solvent was removed and the residual mass of extract was concentrated, dried and for further studies placed in desiccator.



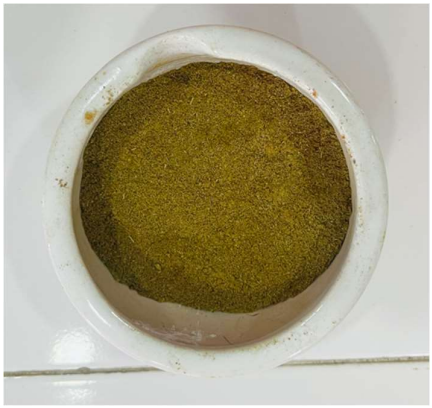

Fig 3 & 4 *Terminalia chebula* fruit powder



Fig 5: Extraction of fruit powder

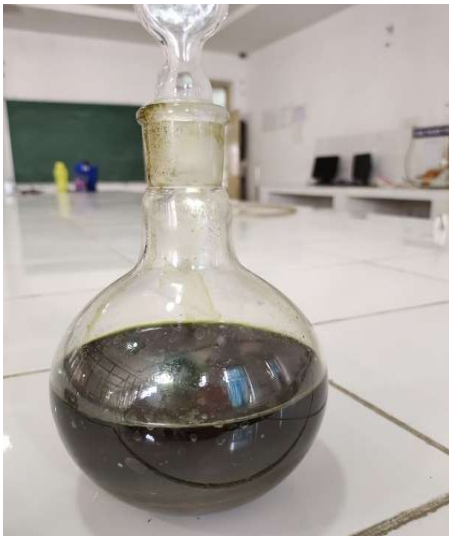

Fig 6: Extract of *Terminalia chebula* by Soxhlet Apparatus

Phytochemical screening

Preliminary phytochemical analysis was performed to detect the presence of various classes of phytochemicals by standard methods. [6] Extracted material of *Terminalia chebula* was subjected to preliminary phytochemical screening to find out various phytochemical constituents like amino acids, steroids, carbohydrates, proteins, glycosides tannins, flavonoids and alkaloids. [7] Tests are given below:

A. Test for alkaloids

Dilute hydrochloric acid was added to the sample. Later, it was vortexed & filtered. Later, successive tests were performed with the extract.

Dragendorff's test

Few drops of Dragendorff's reagent were added to sample, the existence of alkaloids was confirmative by advent of reddish-brown colour.

Mayer's test

4 ml sample was added with Mayer's reagent. Occurrence of alkaloids is directed by advent of white precipitate**.**

Hager's test

4 ml material was added to Hagers reagent. Existence of alkaloids is designated by advent of yellow precipitate**.**

Wagner's test

4 ml sample was added to small quantity of Wagner's solution. Existence of alkaloids is quantified by advent of red-brown precipitate

Test for proteins [8]

Biuret test

2 ml material was added to NaOH 4% and small quantity of 1% solution of $Cuso_4$. Presence of alkaloids is indicated by the non-appearance of violet or pink colour.

Millon's test

4 ml of sample added to million's reagent 5 ml. White precipitate was appeared, upon boiling the precipitate changes to brick red.

Xanthoprotein test

1 ml concentrated sulphuric acid was added to 3 ml of sample, white precipitate is obtained. After boiling, precipitate shown yellow colour. Later ammonium hydroxide was poured and lastly precipitate shown orange colour.

Tests for amino acids

Ninhydrin test

5% ninhydrin solution, 3 drops were mixed to material & boiled for some time. Purple shade not observed.

Test for steroids

4 ml of conc sulphuric acid was added to sample, after shaking chloroform layer shown red & green-yellow fluorescence by acid layer.

Liebermann-Burchard reaction

4 ml chloroform, acetic anhydride mixed to sample & later conc. Sulphuric acid 3 ml were poured through edges of test tube. In the beginning red colour, next blue and in conclusion green colour observed.

Liebermann's reaction

3 ml of acetic anhydride added to few ml of sample. material was warmed and later chilled, lastly conc. Sulphuric acid poured & finally blue colour was observed.

Test for glycosides

Tests for Cardiac Glycosides

Keller Killiani test

Material poured into chloroform 2 ml, later H_2SO_4 was mixed to acquire a layer & at junction colour appeared was noted. Formation of brown ring at junction of 2 layers is distinctive of deoxy sugars in cardenolides. GAA, conc H_2SO_4 & 1drop $Fecl_3$ were mixed with extract. Reddish brown colour observed on joining of 2 layers, and uppermost one looks blue-green, represents occurrence for glycosides.

Saponin glycosides test

Hemolytic test: Hemolytic zone was observed on glass slide when blood was added to drug sample.

Test for foam: Persistent foam was observed when dry powder was dissolved in water.

Tests for flavonoids

Shinoda test: Few ml of ethanol & conc HCl well along 0.5g of magnesium turnings mixed to sample extract. Pink colour was appeared indicating the existence of flavonoids.

G. Tests for tannins

10% lead acetate solution added to material, presence of tannins indicated by the appearance of white precipitate.

Sterols Test

Material mixed to 6% KOH and the sterols presence was indicated by appearance of pink colour.

Determination of Antibacterial activity

Culture of Test Microbes

For the cultivation of bacteria, Nutrient Agar Medium (Beef extract-1.0 g, Yeast extract-2.0 g, Peptone - 5.0 g, NaCl-5.0g, Agar-15.0 g, distilled water 1L) were prepared and sterilized at 15 lbs pressure and 121°C temperature for 25-30 min. Agar rest plates were prepared by pouring approximately 15 ml of Nutrient Agar medium into the Petri dish under aseptic conditions.

Agar Well Diffusion Method

The Methanolic extracts of fruit of *Terminalia chebula* were tested by Agar Well Diffusion method (4 mm) holes were punched aseptically in nutrient agar plate by using a sterilized cork borer. The cotton swabs were dipped into the broth culture of the test organisms and were gently squeezed against the inside of the tube to remove excess fluid. *Staphylococcus aureus* and *Escherichia coli* were swabbed on Agar plates. Swabbing was done in outside diameter of the plates. The plates were allowed to dry for about 5 minutes. Then the extracts of *Terminalia chebula* for four different concentrations (25%, 50%, 75% and 100%) were added into wells of Petri plates. Pure solvents were used as control whereas ampicillin was used as reference for bacterial species. The plates were incubated at 37°C for 24 hrs. The zones of inhibition were measured in millimeters (mm), using Vernier caliper. The Zone size was recorded and all the cultures were discarded by autoclaving. Experiments were performed in triplicates.

RESULTS

Determination of solvent extractive values

The extractive values of *Terminalia chebula* were displayed in Table 1. The powder of *Terminalia chebula* which was air-dried & extracted. The yields of the extract was found to be 7.5% intended for methanol.

Table 1: *Terminalia chebula* extractive values

Plant	Part	% Yields of extracts
		Methanol
Terminalia chebula	Fruit Powder	7.5%

Phytochemical screening

The screening discovered the occurrence of some phytoconstituents like Tannins, flavonoids, alkaloids and steroids were observed in extract of methanol (displayed in Table 2).

Table 2: *Terminalia chebula* phytochemical constituents

S. No	Chemical test	Observation	MeOH
A	**Alkaloids**	Reddish brown precipitate	++
	Dragendorff's	White precipitate	
	Mayer's		
B	**Proteins**	Violet/Pink color	--
	Million's	Orange color	
	Biuret	Red color precipitate	
C	**Amino acids**	Bluish/ Purple color	--
	Ninhydrin		
D	**Steroids**		++
	Salkowski reaction	Yellow Fluorescence	
	Lieberman-Burchard reaction	Green color	
E	**Glycosides**		
	Cardiac glycosides	---	--
	(Keller-Killiani) Test		
F	**Flavonoids**	Pink color	++
	Shinoda test		
G	**Tannins**	White Precipitate	
	Test		++

+ sign indicates present, – sign indicates absent

Antibacterial activity

The antibacterial activity of methanol extract of fruit of *Terminalia chebula* was studied against both gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*) bacteria. The antibacterial activity was compared with standard drug Ampicillin at 10mg/ml concentration. Results were displayed in Table 3,4,5.

Table 3: Antibacterial activity of fruit extract of *Terminalia chebula* by agar-well diffusion method against *Staphylococcus aureus.*

Compound name	Zone of Inhibition (mm)			
	25µL	50µL	75µL	100µL
Methanol extract	0.2	2.4	3.1	4.8



Fig 7: Zone of inhibition of methanol extract against *Staphylococcus aureus*

Table 4: Antibacterial activity of fruit extract of *Terminalia chebula* by agar-well diffusion method against *Escherichia coli*

Compound name	Zone of Inhibition (mm)			
	25µL	50µL	75µL	100µL
Methanol extract	0.9	1.4	2.1	3.0



Fig 8: Zone of inhibition of methanol extract against *E.coli.*

Table 5: Evaluation of Antibacterial activity by Standard drug-Ampicillin

S.NO	Test organism	Standard drugs	Diameter of zone of Inhibition(mm)
1	*Staphylococcus aureus*	Ampicillin	6.2 mm

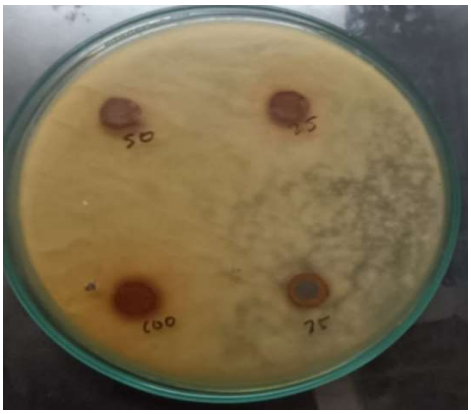

Fig 9: Zone of inhibition by standard drug Ampicillin.

The *T. chebula* fruit extract was highly effective against *Staphylococcus aureus*, and *Escherichia coli.*

DISCUSSION

The extracts were tested for alkaloids, terpenoids, carbohydrates, saponins and tannins, and results are as reported in table 3, and the results of zones of inhibition are reported in table 4 and 5. The zones of inhibition of standard (ampicillin) the zone of inhibition for S. aureus and E. Coli were 6.2 mm and 8.4 mm. The maximum activity was observed at 100% concentration of different extracts of dried fruit powder. Thus, the growth of both S. aureus and E. Coli were inhibited by methanolic extracts of *Terminalia chebula* fruit. The standards of extraction are appreciated to evaluate the chemical ingredients existing in the crude drug & additionally support in assessment of particular ingredients soluble in a specific solvent. Plant constituents show an imperative part in ground of innovative drugs R & D due to their easy availability, low toxicity and cost-effective ness. The bioactive constituents of plants are very imperative. Flavonoids are imperious group of polyphenols extensively scattered amongst the plants. Structurally, they have more than 1 benzene ring in the structure & plentiful information advantage the practice as antioxidants. Existence of several plant ingredients in *Terminalia chebula* might be accountable to diverse pharmacological actions.

CONCLUSION

The investigation had revealed the presence of many secondary metabolites in the extract of *Terminalia chebula*. In the present study the extracts were subjected to antibacterial activity screening using Ampicillin as standard. The results had shown that the methanol extract of fruit of *Terminalia chebula* at 100mg concentrations exhibited a significant antibacterial activity against both Gram positive and negative organisms. The preliminary phytochemical screening revealed the presence of Flavonoids, steroids, tannins and terpenoids. The bioactive compounds present in the fruit is responsible for antibacterial properties of *Terminalia chebula* that form the basis of their use in herbal medicine in India. It has exhibited significant antibacterial activity in comparison to that of standards Ampicillin. These observations of *Terminalia chebula* provide supporting data in the clinical use of various microbial infections.

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