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Phytochemical Analysis and Biochemical Characterization of *Terminalia chebula* Extracts for its Medicinal Use

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Alternative medicine has become popular these days as it is gaining practices across the globe. Our studies aim to detect phytocompounds and biochemical activities of aqueous, dichloro methane, ethyl acetate and methanolic extracts of *Terminalia chebula*. Antioxidant, flurorescence and anitmicrobial studies were carried out for the phytocompounds. Methonolic extracts at 517nm exhibited maximum activity with 82% of free radical scavenging property. Methanolic extract also showed highest antimicrobial activity towards *S.aureus* of 7mm and *S.pyogenes* of 6mm respectiviely. Hence, it can be concluded that *Terminalia chebula* may be a promising candidate in pharmaceuticals and future medicine.

Keywords: *Terminalia chebula*, Combretaceae, DPPH, Phytochemical, Fluorescence.

Plant kingdom has proved to be the most useful in the treatment of many diseases and they provide an important source of all the pharmaceuticals in the world[1]. Plants in all phases of life have served a valuable starting material for drug development[2]. Few of the bioactive constituents of these plants are steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins and glycosides[3]. *Terminalia chebula*, were investigated for anti-lipid peroxidation and free radical scavenging activities[4]. *Terminalia chebula* has been extensively used in ayurveda, unani and homoeopathic system[5]. Studies by fluorescence analysis revealed that *Terminalia chebula* extract at lower concentrations may induce apoptosis[6], while at high dosage, necrosis will lead to death of cell[7]. Silver nanoparticles are observed to have a good catalytic activity on the reduction of methylene blue by *T. chebula* which is confirmed by the decrease in absorbance maximum values[8]. Water extracts of *T. chebula* showed significant antibacterial activity and had a minimum inhibitory concentration (MIC) and minimum bactericidal concentration[9]. *Terminalia chebula* as probiotic exerted a potent inhibitory effect against *C. perfringens* and *E. coli* as indication of at least one of the pharmacological properties of *T. chebula* fruits[10]. *T. chebula* studies in several cancer cell lines showed healing of wounds with improved contraction[11], also significant increase in total protein and DNA of treated wounds[12]. *Terminalia chebula* fruits possessed activity against HIV-1 integrase inhibitors and antimicrobial activity[13] and shown to possess potent intestinal maltase

inhibitory activity[14]. Studies were also explored the activity of *Terminalia chebula* fruit extract on inhibition of growth of strains which are resistant to many antibiotics[15].

MATERIAL AND METHODS

Seed extraction and preparation of smoothie

Loosely packed black karakkaya - *Terminalia chebula* (100g) were procured from Amazon India. Seeds were extracted by grinding and or made into smoothie[16], extract was washed thoroughly with distilled water and then dried under shade conditions. Suspension was mixed for an hour at 37^0C and filtered through Whatman No 1 paper. The filtrate was spin at 14,000 rpm (Thermo, MicroCL 21 Microcentrifuge) in cold conditions for 10 min and supernatant was removed and stored at 4^0C until further analysis.

Phytochemical analysis of *Terminalia chebula*

Seed extract was performed for detection of alkaloids, flavonoids, phenols, carbohydrates, glycosides, terpenoids, saponins, proteins and tannins using standard procedures[17]. The behavior of *Terminalia chebula* with different various chemical reagents using standard methods was assessed. Finally, each extract was dried overnight in a freeze dryer (Ilshin Biobase, Europe - TFD8501) before calculating the yield of each extract. All of the dried extracts were brown solids and were stored at -20°C prior to phytochemical composition analyses and bioassays.

Determination of crude fiber content

To determine the crude fiber content[18], 2g of seed extract was added with 200ml of 1.25% sulphuric acid and boiled for 30min under reflux. It was filtered and washed with boiling water to remove acid. Residual components were rinsed in 1.25% sodium hydroxide solution for 30 min under boiling conditions. To reach neutral state, filtrate was washed with boiling water, dried and calculated with reference drug to obtain constant weight.

Fluorescence Analysis

The fluorescence analysis of the seed extract with various extracts was carried out by using the method of Chase and Pratt[19]. Fluorescence analysis of extract was done by mounting in different solvents which was further analyzed under UV (254 nm and 365 nm) and day light. The behavior of the extract with different solvents was also carried out.

DPPH radical scavenging activity

To 0.1 ml of various extracts of DPPH an equal volume of test compound was added at different concentrations in ethanol. Equal volume of DCM, ethyl acetate, water and ethanol was added to control. Above mixture was kept at room temperature for 20 minutes for incubation. Scavenging capacity was calculated by monitoring the decrease in absorbance at 517 nm[20].

Antimicrobial activity

Bacterial susceptibility towards seed extract was determined by using the standardized minimum inhibitory concentration method. Four bacterial species, *Streptococcus pyogenes, Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* procured from NCIM, Pune were used as test organisms. Inocula were prepared by adding overnight culture of the

Table 1. Phytochemical studies of *Terminalia chebula* extract

S. No	Secondary metabolites	Methanol	Ethyl acetate	Dichloro Methane	Aqueous
1	Alkaloids	+	-	+	-
2	Terpenes	+	+	+	-
3	Saponins	-	-	+	++
4	Steroids	+	+	+	+
5	Carbohydrates	-	-	+	-
6	Flavonoids	+	+	++	+
7	Tannins	+	+	+	+
8	Glycosides	-	-	+	-
9	Polyphenols	+	+	++	+
10	Fixed oil	+	-	+	-

organism in respective broth and were used for antimicrobial activity[21].

RESULTS AND DISCUSSION

Qualitative phytochemical screening of the leaves, revealed that proteins, carbohydrates and tannins were present in both aqueous and methanolic extracts of all the parts. Alkaloids were detected in both aqueous and methanolic extracts of fruit. Flavonoids were present in both type of extracts of all parts except root. Saponins and glycosides were present in all extracts; however glycosides were not detected in Borntrager test. Volatile oil was only detected in the methanolic and aqueous extracts. The result as in table 1 show that these plants rich in bioactive compounds and hence is a potential source of therapeutic properties.

Fluorescence Analysis

Observations were made under visible light and under UV light of short wave length and long wave length. The fluorescence analysis of powder with various reagents and extracts are given in the table 2.

DPPH radical scavenging activity of extracts

Different fractions of *Terminalia chebula* for free radicals of 1, 1-diphenyl 1-2-picryl-hydrazyl (DPPH) showed remarkable scavenging activities in table 3. Methanolic extract showed the highest scavenging activity followed by aqueous extract. DPPH scavenging activity was significantly correlated with phenolics and flavonoids in different extracts.

Table 2. Fluorescence analysis of various extracts

S.No	Extracts	Day light	UV light	
			Short (254nm)	Long (365nm)
1	Methanol	Red	Red	Dark Red
2	Ethyl acetate	Reddish brown	Brown	Dark Red
3	Dichloro Methane	Red	Light Red	Light Red
4	Aqueous	Brownish Red	Orange Red	Light Red

Table 3. Antioxidant activity of root solvent extracts based on their polarity

Extracts	Concentration of extract in PPM	% of DPPH free radical Scavenging activity
Methanol	50	65%
	100	68%
	150	70%
	200	75%
	400	82%
Ethyl acetate	50	45%
	100	58%
	150	60%
	200	65%
	400	68%
Dichloro Methane	50	55%
	100	58%
	150	65%
	200	70%
	400	75%
Aqueous	50	55%
	100	58%
	150	60%
	200	69%
	400	77%

Determination of antibacterial activity

Various extracts of *Terminalia chebula* was tested towards four organisms, *Streptococcus pyogenes*, *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.* Overnight cultures were prepared in Luria broth (LB) media by inoculation with a single colony from agar plates and incubated at 37/ °C for 12/ h. Broth was incubated in the presence of extract compared to the growth of the control culture where only media and bacterial inoculum was taken. The antimicrobial activity index of extracts of *Terminalia chebula roots* at different concentrations was also investigated and is detailed in table 4.

CONCLUSION

Phytochemicals present in the different extracts of roots of *Terminalia chebula* was identified as prominent source for anti-oxidant property. Among the extracts, methanolic extract has highest anti-oxidant property when compared

Table 4. Antibacterial activity of extracts - Values, including diameter of the well (mm), are means of three replicates

Compound No	Gram negative bacteria		Gram positive bacteria	
	E.coli NCIM 2065	*P.aeruginosa* NCIM 5029	*S.aureus* NCIM 5021	*S.pyogenes* NCIM 2608
	Zone of inhibition in mm			
Methanol	4	3	7	6
Ethyl Acetate	3	2	3	5
Dichloro Methane	5	4	2	2
Aqueous	1	2	1	2

to other extracts. In the present study it was found that *Terminalia chebula* methanolic extract has an excellent antimicrobial activity. The pathogenic bacteria were inhibited in presence of the root extracts of *Terminalia chebula* Therefore the future studies should be aimed to exploit this plant to be used as one of the best medicinal plant is controlling pathogenic bacteria.

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