RESEARCH PAPER | Biochemistry | Volume : 4 | Issue : 1 | Jan 2014 | ISSN - 2249-555X

Phytochemical, Antibacterial and Functional Group Identification of Medicinally Useful Plant *Terminalia chebula* Retz., against *Staphylococcus aureus, Psuedomonas aeruginosa* and *Klebsiella pneumoniae*

KEYWORDS: *Terminalia chebula*, Antibacterial activity, Microorganism, FT-IR and Organic analysis.

V. Eugin Amala
Research Scholar, Department of Biochemistry, Govt. Arts College (Autonomous), Kumbakonam, Tamilnadu, India

Dr. M. Jeyaraj
Research Supervisor, Department of Biochemistry, Govt. Arts College (Autonomous), Kumbakonam, Tamilnadu, India

ABSTRACT *Terminalia chebula is known as "Haritaki" belong to the family Combretaceae. It is also known as Mother of all healing. It has antifungal, antibacterial, anticarcinogenic, antidiabetic, antiproliferative, antiarthritic, anticaries and having multiprotective effect such as hepato-protective, pulmonary-protective, renal-protective, radio-protective, cyto-protective and cardio-protective effects. Multiple drug resistance has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. Now a day's multiple drug resistance has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. This situation forced scientists to search for new antimicrobial substances and these fruits are beneficial for the five senses as they improve their receiving powers. Phytochemical screening was done to know the major phytoconstituents present in the plant material, Disc diffusion method was used for antibacterial susceptibility testing, FT-IR and organic analysis also done to identify the functional groups. Hence this results demands further research to unfold its therapeutic values.*

1. Introduction:

Terminalia chebula belong to the family of Combretaceae. Haritaki is also known as "King of medicines" in Tibet. They are listed at the top of the Ayurvedic Materia Medica for its extraordinary healing powers and it is referred as mother of all healing. Hindus use these fruits in worshipping. Traditionally *Terminalia chebula* is used to cure fever, cough, diahorrea, gastroenteritis, skin diseases, candidiasis, urinary tract infection and wound infections. In ayurveda, fruits are used as antimicrobial (1), antioxidant, anti-inflammatory, wound healing, stomachic, laxative, tonic, carminative, expectorant, antihelminthic, antidescentric.

Terminalia chebula have been investigated for potential activity against certain human cancer cell lines (2). In combination with *E. officinalis and T. chebula, T. bellirica* reduced cholesterol-induced atherosclerosis in rabbits (3). Phytochemical analysis of *T. chebula* shows the presence of gallic acid, ellagic acid, tannic acid, ethyl gallate, chebulic acid, chebulagic acid, corilagin, mannitol, ascorbic acid and other compounds (4). One source lists *T. chebula* as having 32% tannin content (5). Thus phytochemical analyses of *T. chebula* extract composition are necessary and provide useful information. The nature of the extracting solvent is the most important factor in the extraction of antioxidants (6). Polar solvents and alcoholic solutions frequently provide satisfactory extract, and the most suitable for plant extractions are methanol, water, and ethanol (7). One of the more alarming recent trends in infectious diseases has been the increasing frequency of antimicrobial resistance among microbial pathogens causing nasacomial and community acquired infections. Commonly found bacteria which causes urinary tract infections includes *Staphylococcus aureus, Psuedomonas aeruginosa, Klebsiella pneumonia* and others (8). The natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanism of action (9). They are effective treatment of infectious diseases, while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (10).

2. Materials and Methods

2.1 Sample Collection and authentication

Fresh fruits were collected from Atthipattu, Thiruvannamalai, Tamilnadu, India. Further it was identified and authenticated with voucher specimen No.VEA/OO1/2013 by Dr. John Britto, Rapinet Herbarium, St. Joseph's College Trichy, Tamilnadu, India. The quantitative estimation of primary metabolites was carried out using various protocols for the analysis of carbohydrate (11), Protein (12) and Lipids (13).

2.2 Preparation of extracts

Terminalia chebula fruits were shade dried, deseeded and pulverized into fine powder using a stainless steel blender. Extracts were prepared by using Soxhlet extractor and 95% ethanol filtrates were individually pooled and each solvent removed at 40°C, under reduced pressure by rotary evaporator. The methanolic, ethanolic and aqueous extracts were subjected to preliminary screening of various plant constituents (14).

2.3 Preparation of Microorganism

The bacterial strains used in this study were gram positive bacteria - *Staphylococcus aureus* (MTCC 3160) gram negative bacteria- *Psuedomonas aeruginosa* (MTCC 1934) and *Klebsiella pneumoniae* (MTCC 4030) procured from MTCC, Chandigarh, India. All chemicals media components and antibiotic impregnated discs were used in this study from Hi Media, Mumbai, India.

2.4 Preparation of Medium

As we procured cultures from MTCC following their suggestion nutrient agar media had been used for *Staphylococcus aureus* and *Psuedomonas aeruginosa,* LB medium for *Klebsiella pneumoniae* these microbes were sub-cultured and used for the antibacterial activity.

2.5 FT-IR analysis

Fourier Transform Infrared Spectroscopy (Themo Scientific Nicolet 1S5 FT-IR Spectrometer) study was carried out in the Department of Chemistry, Annamalai University, Chidambaram, Tamilnadu, India to identify the functional groups present in *Terminalia chebula* adsorbents of 4000-600 cm^{-1} range. The adsorption capacity of adsorbent depends upon porosity as well as chemical reactivity of functional groups at the adsorbent surface (15).

Volume : 4 | Issue : 1 | Jan 2014 | ISSN - 2249-555X

3. Results

The aqueous extract of *Terminalia chebula* was subjected to photochemical screening and revealed the constituents such as alkaloids, flavanoids, saponins, tannins, polysterols, glycosides, carbohydrates, proteins and aminoacids were present. Antibacterial activity against gram negative bacteria *Psuedomonas aeruginosa, Klebsiella pneumonia* and gram positive bacteria *Staphylococcus aureus* also tested and these were susceptible to the selected extract of *Terminalia chebula*. FTIR technique and organic analysis also done to identify the functional groups present in the sample.

Table:1 Phytochemical Screening of *Terminalia Chebula* fruit extract

Phytochemicals	Inference
Test For Alkaloids Wagners Test Mayers Teat	 +++ ++
Test for Flavanoids Alkaline Reagent Test	 ++
Test for Carbohydrates Molisch's Test	 +++
Test for Glycosides Legal's Test	 +
Test for Saponins Reaction with lead Acetate Test	 ++
Test for Tannin Ferric Chloride Test Lead Acetate Test Reaction with Potassium Dichromate	 +++ + ++
Test for Proteins and Aminoacids Ninhydrin Test Biuret Test	 + +
Polyphenols Ferric Chloride & Potassium Ferric Cyanide	 +++
Test for Quinone Reaction with Sodium Hydroxide	 +
Test for Caumarin Reaction with Sodium Hydroxide	 +

Table:2 Macromolecules of *T. chebula* from various solvents

Solvents	Acetone (mg/dl)	%	Methanol (mg/dl)	%	Aqueous (mg/dl)	%	$\bar{x}$	
Carbohydrate	17.7	28	21.3	34	24.36	38	21.12	2.72
Protein	12.7	29	10.3	24	20.7	47	14.57	4.44
Lipids	10.3	26	12.7	31	17.2	43	13.40	2.86

Table:3 Antibacterial activity of *Terminalia chebula* from various solvents

Test Samples and Extracts	Zone of Inhibition (mm)							
	Gram Positive	%	Gram Negative				$\bar{x}$	
	S. aureus	%	P. aeruginosa	%	K. pneumoniae	%		
Acetone	10.00	33.05	10.25	33.88	10.00	33.05	10.08	0.12
Methanol	10.75	27.38	10.75	27.38	17.75	45.22	13.08	3.30
Aqueous	10.00	10.91	11.00	33.58	11.75	35.87	10.92	0.72
Antibiotic	10.00	23.25	14.00	32.55	19.00	44.19	14.3 3.68	

Table:4 FTIR peaks of *Terminalia chebula* fruit extract

Functional Group	OH Alcohol	CH Alkane	C=O Carbonyl Group	NH Amide	NO Nitro	C=C Aromatic	CF Alkyl Halide	=C-H Alkene
Wavelength	3387.25	2925.79, 2855.07	1778.51, 1721.09, 1710.01	1619.03	1534.7	1466.22 1477.05	1345.02, 1209.24, 1034.67	871.78, 764.95, 671.81

Fig:1 FTIR study for KBr absorption from 600-4000nm

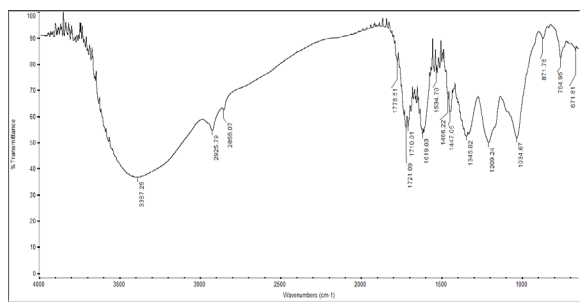

Table:5 Systematic organic analysis for functional groups

S.No	Experiment	Observation	Inference
1	Aromaticity	Smoky flame	Aromatic compounds
2	Solubility	Soluble in $NaHCo_3$	Acidic
3	Test for Unsaturation	Decolorized with precipitation	Phenol or aromatic amine
4	Sodium Fusion Test	Green colour	Nitrogen
5	Test for Acids	Red colour	Acid
6	Test for Phenol	Dark green colour	Phenol
7	Test for Carbonyl compound	Yellow precipitate	Aldehydes or Ketones
8	Fehling's Test	Reddish brown precipitate	Aldehyde
9	Tollen's Reagent Test (Legal Test)	Purple colour.	Ketone
10	Test for Esters	White precipitate	Presence of ester or aromatic acid
11	Test for amine	Red ring formation	Aromatic primary amine
12	Test for amide	Smell of Ammonia	Aromatic Amide
13	Test for diamide	Violet colour	Diamine (Urea)
14	Molisch's Test	Deep violet colour	Carbohydrate

4. Discussion

Terminalia chebula has the phytoconstituents such as tannin, flavanoids, alkaloids, carbohydrate, glycosides, saponins, protein, aminoacid, polyphenol, quinine, and caumarin have shown in Table:1 describes the degree of present carbohydrate, tannin and alkaloids were remarkably present. Tannin content of *Terminalia chebula* largely depends on its geographical location (16). Macromolecules explicitly carbohydrate, protein and lipids were tested with methanol, acetone and aqueous quantitatively and it is displayed in

Volume : 4 | Issue : 1 | Jan 2014 | ISSN - 2249-555X

Table:2. Among that aqueous extract has higher percentage and standard deviation of carbohydrate, protein and lipids such as 38%, 47% and 43% and 2.72, 4.44, 2.86 respectively. Sugars are reported to possess freeze resistance in plants (17). Protein hydrolyses from various sources and reported to possess antioxidant activity (18). Lipid has been used in compounding of pharmaceutical and cosmetic preparations. Various lipids and related compounds have been extensively studied for their growth inhibitory effect on pathogenic strains (19).

Table:3 shows the extract of *T.chebula* have activity on *S.aureus, P.aeruginosa* and *K.pneumoniae*. From that strongest inhibitory zones are *Klebsillae pneumoniae* Methanol (17.75mm), aqueous(11.75mm) and aqueous *P.aeruginosa*(11mm) and its percentage 45.22%, 35.87%, 33.58% correspondingly. From that methanol extract has more efficiency than the other solvents, the extract of *Terminalia chebula* showed broad spectrum of antibacterial activity (20). In order to evaluate the functional group FTIR technique has done in the corresponding retention time is shown in table:4 and Figure:1. *T.chebula* has the functional groups such as OH, CH, C=O, NH, C=C, CF, =CH respectively and it is again confirmed with organic analysis which is shown in Table:5 and Table:1 polyphenols, Tannin, carbohydrate are richly present with the peak of 3387.25 cm^{-1}, 1778.51 cm^{-1}, 1721.09 cm^{-1} and 1710cm^{-1}.

5. Conclusion

From the above study we conclude that different fractions of *Terminalia chebula* were found to inhibit the growth of different types of organisms in a very selective manner. Therefore further studies on the extract are needed to pinpoint the findings. This report may serve as a footstep on this aspect to evaluate organelle protective effect with the aid of animal models.

REFERENCE 1. Sudarshan S R. Encyclopedia of Indian Medicine, Mumbai:Popular Prakashan;2005;4:92-93 | 2. Saleem A, Husheem M, Härkönen P, Pihlaja K. Inhibition of cancer cell growth by crude extract and the phenolics of Terminalia chebula retz. fruit . J Ethnopharmacol . 2002;81:327-336. | 3. Thakur CP, Thakur B, Singh S, et al. The Ayurvedic medicines Haritaki, Amala, and Bahira reduce cholesterol-induced atherosclerosis in rabbits. Int J Cardiol . 1988;21:167-175. | 4. I.S.Grover and S. Bala, "Antimutagenic activity of T. chebula (myroblan) in Salmonella typhimurium,"Indian Journal of Experimental Biology, vol. 30, no. 4, pp. 339–341, 1992. | 5. R.R.Chattopadhyay and S.K.Bhattacharyya, "Plant review Terminalia chebula: an update,"Pharmacognosy Reviews, vol.1, no. 1, pp. 151–156, 2007. | 6. I.Hinneburg and R. H. H. Neubert, "Influence of extraction parameters on the phytochemical characteristics of extracts from buckwheat (Fagopyrum esculentum) herb," Journal of Agricultural and Food Chemistry, vol. 53, no. 1, pp. 3–7, 2005. | 7. B. Sultana, F. Anwar, and M. Ashraf, "Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts," Molecules, vol. 14, no. 6, pp. 2167–2180, 2009. | 8. Idowu AO, Odelola HA. Prevalence of some Uropathogenic Bacterial Isolates and their susceptibility to some Quinolones. African J Biomed Res 2007;10:269-273 | 9. Ahmad I, Aqil F. Invitro efficacy of bioactive extracts of 15 medicinal plants against ESbetaL-producing multidrug-resistant enteric bacteria. Microbial Res2007;162:264-75. | | | 10. Iwu MW, Duncan AR, Okunji CO. New microbials of plant origin In: Perspective onnew crops and new uses, In: Janick J editor. Alexandria, VA:ASHS Press; P.1999:457-62 | 11. Dubois M., Gills, K.A.,Hamilton, J.K.,Rebers, P.A. and smith, F.1956. Colorimetric method for determination of sugar and related substances. Anal. Chem.28:350-356 | 12. Lowry O.H., Rosebrough, N.J., Far, A.L. and Randall, R.J.1951. Protein measurement with the Folin-phenol reagent.J.Biol.Cbem.193:263-275 | 13. Jayaraman J.1981.Laboratory manual in biochemistry. Wilsey Eastern Ltd, New Delhi.pp 96-97 | 14. Kokate C.K., 2001, Practical Pharmacognosy, Vallabh Prakashan, pp. 218. 16. Mohammed A., 1994, Text Book of Pharmacognosy, CBS Publishers and Distributors... | 15. Kumar P.S, Ramalingam S, Senthamarai C, Niranjanaa M, Vijayalakshmi P, Sivanesan .S(2010) Adsorption of dye from aqueous solution by Cashewnut shell: Studies on equilibrium isotherm, kinetics and thermodynamics of interactions. Desalination, 261:52-60. | 16. Kumar K.J. Effect of Geographical variation on contents of Tannic acid... in Terminalia chebula. Natural products 2006;2(3-4):170-175. | 17. Ashworth E.N., V.E. and Volenec, J.J.1993. Seasonal variations in soluble sugars and starch within woody stems of Cormssericea L. Tree Physiology. 13:379-388. | 18. Pena-Ramos, E.A. and Xiong, Y.L.2003. Whey and soy protein hydrolysates inhibit lipid oxidation in cooked pork patties. Meat sci.64:259-263 | 19. Meng J.C.,Zhu, Q.X. and Tan, R.X.2000. New antimicrobial mono and sesquiterpenes from Soroseris hookeriana subsp. Erysimoides. Plant Medica.66:541-544 | 20. Phadke, S.A, Kulkarni, S.D, 1989. Screening of invitro antibacterial activity of Terminalia chebula, Eclipta alba and Ocimum sanctum. Indian journal of Medical sciences, 43(5):113-117 |