PharmacologyOnLine

Archives • 2021 • vol.2 • 43-54

PHYTOCHEMICAL ANALYSIS OF ETHANOLIC EXTRACT OF FRUITS OF TERMINALIA CHEBULA AND ITS MEDICINAL USE IN HUMAN

R. Arbind Kumar Choudhary,[1] E. Manivannan,[1] Chandrashekar R[2], Indla Ravi [1] V.Sivasankari, [1], Arul Kothai Arul,3

1. Department of Pharmacology, Vinayaka Mission's Kirupananda Variyar Medical College and Hospitals, Sankari Main Road, Seeragapadi, Salem..
2. Department of Pharmacology, A.J. Institute of Medical Sciences and Research Centre, Mangalore-Karnataka, India
3. Dept.of Pharmacology, VM College of Pharmacy, Salem.

* arbindkch@gmail.com

Abstract

Terminalia Chebula has been extensively used in field of traditional medicine and AYUSH. Recent Studies using fluorescence analysis given a sense of understanding of Terminalia Chebula fruit pulp**.** Present study is to carry out preliminary phytochemical screening of EEFTC fruit pulp and the Medicinal values . Terminalia Chebula dried fruit The ethanolic extracts were concentrated in Roto flash evaporator and dried and stored in freezer for further biochemical analysis by using screening methods like preparation of smoothie, Phytochemical analysis, evaluation of determination of crude fibre content and antimicrobial activity of plant extracts, Qualitative Phytochemical analysis , we found that the, aqueous , ethanolic extract of T. Chebula (EETC) is rich with all the compound like Phytosterols, Triterpenoids, Carbohydrate, Glycosides, Phenolic compound . The antimicrobial activity index of extracts of Terminalia Chebula dried fruit pulp at different concentrations was also investigated , the antimicrobial activity, indicated by an inhibition zone surrounding the well containing the extract, was recorded if the zone was greater than 8 mm. Terminalia Chebula contains biomolecules which already shown the have anti-viral, anti-inflammatory, anti-oxidant properties, against many pathogen microorganism . in present situation of pandemic outbreak, an expedited screening of therapeutically bioactive constituents is required to establish the anti-SARS-CoV-2 property of these herbs.

Keywords: *Terminalia Chebula1, pathogenic strains2, fluorescence analysis 3, scavenging assay 4, anti-SARS-CoV-2, 5*

Introduction

Natural products have high structural diversity and unique pharmacological or biochemical properties due to the natural selection and evolutionary processes that have made the utility over era.[1] The Medicinal diversity of natural products far exceeds the capabilities of synthetic organic compound within the laboratory. When natural products have been utilized in both traditional and modern medicine for treating Pathological condition[2]. Recently , natural products are often used as starting points for drug discovery lead optimization followed by synthetic modifications to help reduce side effects and increase bioavailability. The natural products are the inspiration for approximately two third of Drug approving authorities drugs.[3] In addition to medicine, natural products and their derivatives are used as food additives in the form of spices and herbs, antibacterial agents, and antioxidants to protect food freshness , teats enhancer[4]. Few of the bioactive constituents of these plants are steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins and glycosides, Terminalia chebula, were investigated for anti-lipid peroxidation and free radical scavenging activities[5]. Terminalia chebula has been extensively used in field of traditional medicine and AYUSH. Recent Studies using fluorescence analysis given a sense of understanding of Terminalia chebula fruit pulp extract at lower concentrations may induce apoptosis, and on high dosage, necrosis will lead to death of malignant cancer cell on laboratory animals [6]. Some Silver nanoparticles where observed to have a good catalytic activity on the reduction of methylene blue by T. chebula which is establishes by the decrease in absorbance maximum values.[7]. Water ethanolic fruit pulp extracts of T. chebula fruit pulp extract has showed significant antibacterial activity, antiviral at minimum inhibitory concentration (MIC) and minimum bactericidal[8]. Terminalia chebula fruit pulp ethanolic extract as probiotic exerted a potent inhibitory effect against , some gram positive , C. perfringens and E. coli indication of at least one of the pharmacological properties[9] .T. chebula fruits ethanolic extract studied on several cancer cell lines showed very effective complementary treatment of superficial cancer and healing of wounds with improved contraction, also significant increase in total protein and DNA of treated wounds and also it is significantly arrest the bacteria found on site of wound like S. aureus followed by E. coli , Proteus, Pseudomonas and Klebsiella The reasons for the differences in antimicrobial drug–resistant patterns might be related to infection control practices or to timing of the introduction of resistant organisms. However, more research is needed to clarify these differences [10, 11] . Terminalia chebula The fruit is used for medicinal purpose . It is considered to be a rasayana or Elixir for Vata, balances tridoshas , enhances digestion (dipanapachana), sharpens the senses displays alterative , astringent, expectorant, anti-inflammatory, anodyne, cardiotonic, laxative, antiseptic and antiemetic properties. Seven different types of fruits are recognized (i.e. vijaya, rohini, putana, amrita, abhaya, jivanti and chetaki), based on the region of harvestation, as well as its colour and shape. [12]

However, the phytoconstituents analysis of species of Terminalia Chebula has not been studied extensively so far and hence the main aim of present study is to carry out preliminary phytochemical screening of EEFTC fruit pulp.

Methods

Terminalia chebula dried fruit (500g) in five pack of 100g were procured from Cloudtail , amazon India, India Private Limited *GMR Airport City, Survey No. 99/1, Mamidipally Village, Shamshabad Hyderabad, Telangana, 500108, The ethanolic extracts were concentrated in Roto flash evaporator and dried and stored in freezer for further biochemical analysis. The extracts were used for the preliminary

phytochemical screening using the following tests, fluorescence analysis of plant powder, DPPH radical scavenging assay

1. SEED EXTRACTION AND PREPARATION OF SMOOTHIE

Terminalia Chebula whole Fruit contenting seeds were extracted by grinding and or made into smoothie and extract was washed thoroughly with distilled water and then dried under shade conditions. Suspension was mixed for an hour at 370C and filtered through Whatman No 1 paper. The filtrate ware spin at 14,000 rpm (Thermo, MicroCL 21 Microcentrifuge) at cold conditions for 10 min . Supernatant ware removed and stored at 40C until further analysis .

2. Phytochemical analysis of Terminalia chebula:

Terminalia chebula Fruit contenting seeds extract chemically analysed for detection of alkaloids, flavonoids, phenols, carbohydrates, glycosides, terpenoids, saponins, proteins and tannins using standard procedures. Terminalia chebula fruit extract with 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.
Following Phytochemicals Analysis was:
Phytochemical analysis of the test solution was done according to published methods biochemicals reaction.

a. TEST FOR PHYTOSTEROLS

Salkowski reaction:

To 0.5 ml chloroform extract in a test tube add 1ml of Conc.H2SO 4 from the sides of the test tube. Appearance of reddish brown colour in chloroform layer indicates presence of phytosterols

b. TEST FOR TRITERPENOIDS

Brieskorn and Binar test:

To chloroform extract, add few drops of chlorosulphonic acid in glacial acetic acid. Appearance of red colour within five minutes indicates presence of triterpinoids.

c. TEST FOR SAPONINS

Foam test:
A small amount of extract taken in a test tube with little quantity of water. Shake vigorously. Appearance of foam persisting for 10 minutes indicates presence of saponins.

d. TEST FOR ALKALOIDS

Dragendroff's test
Dissolve various extract of the herbal drug in chloroform. Evaporate chloroform and acidify the residue by adding few drops of Dragendroff's reagent (Potassium Bismuth Iodide). Appearance of orange red precipitate indicates presence of alkaloids.

e. TEST FOR CARBOHYDRATES

Molisch's test
Mix the extract with Molisch reagent and add Conc. H2SO4 along the sides of the test tube to form layers. Appearance of reddish violet ring the interference indicates the presence of carbohydrates.

f. TEST FOR FLAVANOIDS

Ferric chloride test
To the alcoholic solution of the extract add few drops of neutral ferric chloride solution. Appearance of green colour indicates presence of flavanoids.

g. TEST FOR LACTONES

Legal's test

To the extract mixtures add sodium nitroprusside and pyridine. Then the mixture is treated with NaOH. Appearance of deep red colour indicates the presence of lactones.

h. TEST FOR PHENOLIC COMPOUNDS AND TANNINS FERRIC CHLORIDE TEST

Take 2 ml of extract in a test tube and add ferric chloride solution drop by drop. Appearance of bluish black precipitate indicates presence of phenolic compounds and tannins.

i. TEST FOR PROTEINS

Ninhydrin test

Few drops of Ninhydrin added to the extract. Appearance of blue colour indicates presence of amino acid where as proteins may rarely give positive result.

j. TEST FOR GLYCOSIDES

Keller-Killiani test

Extract+ 1ml of glacial acetic acid + few drops of ferric chloride solution + Conc. H_2SO_4 (Slowly through the sides of the test tube). Appearance of reddish brown ring at the junction of the liquids indicates the presence of deoxysugars

k. SPOT TEST

Prepared spot on the filter paper with the test solution and oil staining on the filter paper indicated the presence of fixed oil & fats.

3. DETERMINATION OF CRUDE FIBRE CONTENT:

To determine the crude fiber content , 2g of dry fruit extract was added with 200ml of 1.25% sulphuric acid solution and boiled for minimum 30min under reflux. Filtered and washed with boiling water to remove unwanted acid content . Residual components were rinsed in 1.25% sodium hydroxide solution (NAOH) for 30 min under boiling conditions. To reach neutral state, filtrate were washed with boiling water, dried and calculated with reference drug to obtain constant weight.

4. FLUORESCENCE ANALYSIS OF PLANT POWDER

The fluorescence analysis of the fruit extract of T. Chebula with various extracts was carried out by using the method of Chase and Pratt. 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 followed by , The behaviour of the T. Chebula extract with different solvents was also carried out.

5. INVITRO DPPH RADICAL SCAVENGING ASSAY

Free radical scavenging activity of different extracts of dry fruit extract of T. Chebula plant were measured. 1, 1- diphenyl-2-picryl hydrazyl (DPPH). 0.1 mM solution of DPPH in ethanol were prepared. This solution (1 ml) was added to 3 ml. of different extracts in ethanol at different concentration (5, 10, 15, 20, 25, 30 µg/ml), dry fruit extract of T. Chebula of DPPH an equal volume of test compound was added at different concentrations in ethanol. Equal volume of Dichloromethane (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 .

6. ANTIMICROBIAL ACTIVITY OF PLANT EXTRACTS

The tested bacterial strains isolates obtained from NCCS, Pune, India. The bacterial stock was maintained at 4 °C on nutrient agar slants. Bacterial susceptibility towards fruit pulp extract was determined by using the standardized minimum inhibitory concentration(MIC) method. Six bacterial species like Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli, and bacteria found on site of wound like Pseudomonas and Klebsiella and Pseudomonas aeruginosa procured from NCIM and Department of microbiology GEMCH Erode , Inoculation were prepared by adding overnight culture of the organism in respective bouillon were used for antimicrobial activity.

Results

The result as in table 1 shown that these T. Chebula fruit rich in bioactive compounds and hence is a potential source of therapeutic properties.

FLUORESCENCE ANALYSIS USING ELECTROMAGNETIC SPECTROSCOPY:

Fluorescence analysis of the fruit extract having medicinal properties was observed under day and UV light using various solvent extracts as well as acids and alkaline treated with solutions of the compound. The extract powder was treated with neutral solvents like hexane, benzene, chloroform, methanol, ethyl acetate, alcohol, acetone and acids like 1N Hydrochloric acid, 50% Sulphuric acid and alkaline solutions like aqueous and alcoholic 1N NaoH , The fluorescence analysis of powder with various reagents and extracts are presented in the Table 2.

DPPH RADICAL SCAVENGING ACTIVITY:

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 3. Antioxidant activity of of T. Chebula fruit solvent extracts based on their polarity:

DETERMINATION OF ANTIBACTERIAL ACTIVITY:

Dried fruit extracts of Terminalia chebula were microbiologically tested for six organisms, Six bacterial species like Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli, and bacteria found on site of wound like Pseudomonas and Klebsiella 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 were incubated in the presence of aqueous extract compared to the growth of the control culture where only media and bacterial inoculum was tested . The antimicrobial activity index of extracts of Terminalia chebula dried fruit pulp at different concentrations was also investigated , the antimicrobial activity, indicated by an inhibition zone surrounding the well containing the extract, was recorded if the zone was greater than 8 mm

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

Discussion

Qualitative Phytochemical analysis , we found that the, aqueous , ethiyl acetate ethanolic extract of T. Chebula(EETC) and dichloro methane possess Phytosterols, Triterpenoids, Carbohydrate, Glycosides, Phenolic compound and Tannins. glycosides were not detected in Borntrager test but Keller-Killiani test we found the active presence , by preforming test , Extract+ 1ml of glacial acetic acid + few drops of ferric chloride solution + Conc. H_2SO_4

(Slowly through the sides of the test tube). Observation of reddish brown ring at the junction of the liquids indicates the presence of deoxysugars and the formation of an orange red precipitate indicates the presence of reducing sugars. Volatile oil ware only detected in the Ethanolic extract and aqueous extracts[12] . Flavonoids play a vital role in protection against human diseases like lipid peroxidation involved in atherogenesis, thrombosis, carcinogenesis, hepatotoxicity, and a variety of disease conditions[13].

In our preliminary phytochemical analysis we found that the EEFTC possess Phytosterols, Triterpenoids, Carbohydrate, Glycosides, Phenolic compound and Tannins. Traditionally sapponins is use for detergents, pesticides as well as mollucicides, some extended is used industrial application such as foaming, surface active agents etcAnd also found to have beneficial medicinal effects [14]. The important role of tannins is to protect from predation, pesticides and also in plant growth regulation. Most of the studies by various other researcher shown that flavanoids provide health benefits through cell signaling pathways and antioxidant effects. Tannins, in particular, ellagitannins, is endogenous inhibitors of the growth of numerous species of pests. They act as an antibiotic substance they have anti-nutritional deterrent against insects and aphids[15].

Fluorescence analysis, Fluorescent characteristics of powdered plant fruit extract with different chemical reagents were determined under ordinary and ultraviolet light according to the procedure given by Kokoshi et al.10 mg of the formulation was taken in a glass slide and treated with various reagents for the presence of their fluorescence characteristics under ultra-violet lamp at 254 and 366 nm. In presence of day light on normal wave length 254nm aqueous of T.ch extract shows brownish reflection where as long wave length ie 365nm shows lighter reddish. Similar observation found with Ethyl acetate, Ethanolic extract, and Dichloro Methane[16].

Extensive use of DPPH radical scavenging activity has been observed for screening antioxidants from Terminalia Chebula fruit juices or extracts . In a study done to understand antioxidant effect radical scavenging activity . In the present study, the results of DPPH radical scavenging activity of ethanolic extract increase in concentration representing a dose-dependent effect . The antioxidant and radical scavenging properties of plants are based on their medicinal value. Results in this study coincide with the results of ethanolic extracts of Terminalia Chebula fruit[17] significant enhancement in the antioxidant potential of Terminalia Chebula when used in a mixture with other extracts is supported by the study which states that several plant preparations such as a mixture of aqueous .

Among all the tested bacterial pathogens sample gram-positive bacterial strains have been found to be more susceptible than gram-negative bacterial strains. This may be because of cellular strictures of the gram-positive like cell wall of gram-positive bacteria made up of a single layer, whereas, the gram-negative cell wall is a multi-layered structure bounded by an outer membrane, Gram-positive bacteria cell wall is thicker comparatively to thin cell wall of Gram-negative bacteria. A majority of the described antimicrobial effects of T. chebula extracts have many secondary metabolites, like tannins. The antibacterial activities of tannins are already established and are known to inhibit the growth of many bacteria,yeast,and viruses of fungi. Terminalia Chebula fruit have demonstrated antibacterial activity against clinical strains of selected microorganisms. [18]The fruit extract shows activity profile. As the fruit extract is mixture of several constituents, it exerts good activity profile. The basis of varying degree of sensitivity of test organism is due to the intrinsic tolerance of

microorganism , chemical nature and structure of the constituent for the mode of action on the control of growth of microorganism is beneficial. The plant has been used in curing various ailments in India; [20]hence the phytoconstituents is useful to develop the molecules against infectious diseases[19]. T. Chebula fruit has shown the better activity profile against gram positive and gram negative bacteria especially against S. aureus.

There is need of extensive reviews exploration some species of plant like Terminalia chebula. The fruit Terminalia chebula of possess significant medicinal values like immunomodulatory effect, due to the presence of some alkaloid, coumarin, flavon, steroids etc. It has got antimicrobial effects include anti-bacterial, anti-fungal, anti-protozoa, anthelmintic, anti-salmonella effects[21]
Effective DPPH scavenging shown its invitro antioxidant activities , which may be due to high polyphenol and tannins contents. The fruit extract also possess marked anti-hypertensive, hypolipidemic effects in various in vivo cardiovascular experiments. Dried pericarp of Terminalia chebula extract contains several alkaloid, flavone, coumarin, steroids etc[22]and its thyl acetate extract showed significant hepatoprotective activity.

The Terminalia chebula and its phytoconstituents have shown arrest of abnormal cancer cell growth , which are responsible for such valuable therapeutic effects s mentioned above. So the current research of phytochemical analysis and biochemical analysis proven the medicinal properties Terminalia chebula . in recent time we found existing antibiotics are becoming resistant against many pathogenic microorganisms, such studies should highly be encouraged to explore the alternative use and maybe possible alternative sources for future antibiotics. Results of the study reveal that T. chebula can be potential candidate to be explored for future. Invitro research of Haritaki [23] (T. chebula) against SARS-CoV-2 may need to screen . Some species of plant Terminalia chebula contains biomolecules which already shown the have anti-viral, anti-inflammatory, anti-oxidant properties, against many pathogen microorganism . In present situation of pandemic outbreak, an expedited screening of therapeutically bioactive constituents is required to establish the anti-SARS-CoV-2 property of these herbs.

Acknowledgments

I take this opportunity to express my profound gratitude and deep regards to my teachers Prof. Dr L Madhan for their exemplary guidance, monitoring and constant encouragement throughout the research of this project. The blessing, help and guidance given by them time to time shall carry me a long way in the journey of life on which I am about to embark.

CONFLICT OF INTEREST: The authors have no conflicts of interest regarding this investigation.

Reference :

1. Dias DA, Urban S, Roessner U. A historical overview of natural products in drug discovery. Metabolites. 2012;2(2):303-336. Published 2012 Apr 16. doi:10.3390/metabo2020303
2. Mishra B.B., Tiwari V.K. Natural products: An evolving role in future drug discovery. Eur. J. Med. Chem. 2011;46:4769–4807. doi: 10.1016/j.ejmech.2011.07.057.
3. Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, et al. Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnol Adv. 2015;33(8):1582-1614. doi:10.1016/j.biotechadv.2015.08.001
4. Yuan H, Ma Q, Ye L, Piao G. The Traditional Medicine and Modern Medicine from Natural Products. Molecules. 2016 Apr

29;21(5):559. doi: 10.3390/molecules21050559. PMID: 27136524; PMCID: PMC6273146.

5. 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. Phytother Res. 2020 Oct;34(10):2518-2533. doi: 10.1002/ptr.6702. Epub 2020 Apr 20. PMID: 32307775.
6. Afshari AR, Sadeghnia HR, Mollazadeh H. A Review on Potential Mechanisms of Terminalia chebula in Alzheimer's Disease. Adv Pharmacol Sci. 2016;2016:8964849. doi:10.1155/2016/8964849
7. T. Jebakumar Immanuel Edison, M.G. Sethuraman, Instant green synthesis of silver nanoparticles using Terminalia chebula fruit extract and evaluation of their catalytic activity on reduction of methylene blue, Process Biochemistry,Volume 47, Issue 9,2012,
8. Bag A, Bhattacharyya SK, Chattopadhyay RR. The development of Terminalia chebula Retz. (Combretaceae) in clinical research. Asian Pac J Trop Biomed. 2013;3(3):244-252. doi:10.1016/S2221-1691(13)60059-3
9. Sharma C, Aneja KR, Kasera R, Aneja A. Antimicrobial potential of Terminalia chebula Retz. fruit extracts against ear pathogens. World J Otorhinolaryngol 2012; 2(2): 8-13 [DOI: 10.5319/wjo.v2.i2.8]
10. Kim, H & Cho, Jin-Ho & Jeong, EY & Lim, J & Lee, Su-Kyoung & Lee, H.s. (2006). Growth inhibitory activity of active component of Terminalia chebula fruits against intestinal bacteria. Journal of food protection. 69. 2205-9. 10.4315/0362-028X-69.9.2205.
11. Swanya Yakaew, Khwunjit Itsarasook, Jatuporn Ngoenkam, Arum Jessadayannamaetha, Jarupa Viyoch & Malyn Ungsurungsie (2016) Ethanol extract of Terminalia chebula fruit protects against UVB-induced skin damage, Pharmaceutical Biology, 54:11, 2701-2707, DOI: 10.1080/13880209.2016.1179768
12. Kancherla N, Dhakshinamoothi A, Chitra K, Komaram RB. Preliminary Analysis of Phytoconstituents and Evaluation of Anthelminthic Property of Cayratia auriculata (In Vitro). Maedica (Bucur). 2019;14(4):350-356. doi:10.26574/maedica.2019.14.4.350
13. Grassi D, Desideri G, Ferri C. Flavonoids: antioxidants against atherosclerosis. Nutrients. 2010;2(8):889-902. doi:10.3390/nu2080889
14. Kumar, Neethu & Nair, Arun & Murali, Megha & Devi P S, Sreeja. (2017). Qualitative phytochemical analysis of triphala extracts. 6. 248-251.
15. Sharma C, Aneja KR, Kasera R, Aneja A. Antimicrobial potential of Terminalia chebula Retz. fruit extracts against ear pathogens. World J Otorhinolaryngol 2012; 2(2): 8-13 [DOI: 10.5319/wjo.v2.i2.8]
16. Gayathri V and Kiruba D: Fluorescence Analysis of Two Medicinal Plants – Psidium Guajava L and Citrus Aurantium. Int J Pharm Sci Res 2015; 6(3): 1279-82.doi: 10.13040/IJPSR.0975-8232.6 (3).1279-82.
17. Hazra B, Sarkar R, Biswas S, Mandal N. Comparative study of the antioxidant and reactive oxygen species scavenging properties in the extracts of the fruits of Terminalia chebula, Terminalia belerica and Emblica officinalis. BMC Complement Altern Med. 2010;10:20. Published 2010 May 13. doi:10.1186/1472-6882-10-20
18. Zhang, XR., Kaunda, J.S., Zhu, HT. et al. The Genus Terminalia (Combretaceae): An Ethnopharmacological, Phytochemical and

Pharmacological Review. Nat. Prod. Bioprospect. 9, 357–392 (2019).

19. Shankara, BE & Ramachandra, Y L & Rajan, S & Preetham, J & Ganapathy, Sujan. (2012). In vitro antibacterial activity of Terminalia chebula leaf gall extracts against some human pathogenic strains. International Current Pharmaceutical Journal. 1. 10.3329/icpj.v1i8.11254.
20.
21. Denis Swolana, Małgorzata Kępa, Agata Kabała-Dzik, Radosław Dzik, Robert D. Wojtyczka. Sensitivity of Staphylococcal Biofilm to Selected Compounds of Plant Origin. Antibiotics 2021, 10 (5) , 607. https://doi.org/10.3390/antibiotics10050607
22. Na, MinKyun & Bae, KiHwan & Kang, Sam & Min, Byung & Jae Kuk, Yoo & Kamiryo, Yuko & Senoo, Yu-ichiro & Yokoo, Seiichi & Miwa, Nobuhiko. (2004). Cytoprotective effect on oxidative stress and inhibitory effect on cellular aging ofTerminalia chebula fruit. Phytotherapy research : PTR. 18. 737-41. 10.1002/ptr.1529.
23. Silveira Dâmaris, Prieto-Garcia Jose Maria, Boylan Fabio, Estrada Omar, Fonseca-Bazzo Yris Maria, Jamal Claudia Masrouah, Magalhães Pérola Oliveira, Pereira Edson Oliveira, Tomczyk Michal, Heinrich Michael, Is There Evidence for the Use of Herbal Medicines as Adjuvant Symptomatic Therapy. Frontiers in Pharmacology, VOLUME=11 , YEAR=2020 PAGES=1479.

Table 1.

s.no	Secondary metabolites	Aqueous	Ethyl acetate	Ethanolic extract	Dichloro Methane
1.	Alkaloids	-	-	+	+
2.	Terpenes	-	+	+	+
3.	Saponins	++	-	-	+
4.	Steroids	+	+	+	+
5.	Carbohydrates	-	-	-	+
6.	Flavonoids Tannins	+	+	+	++
7.	Tannins	+	+	+	+
8.	Glycoside	-	-	-	+
9.	Polyphenols	+	+	+	++
10.	Fixed oil	-	-	+	+

Phytochemical analysis of EEFTC

Table 2.

S.no	Extracts	Day light	UV light
		Normal wave (254nm)	Long-wave(365 nm)
1.	Aqueous	Brownish	Light Red
2.	Ethyl acetate	Reddish lighter	Deep red
3.	Ethanolic extract	Red	Deep red
4.	Dichloro Methane	Red	Light Red

fluorescence analysis under day and UV light

Table 3. Antioxidant activity of of T. Chebula fruit solvent extracts based on their polarity:

Extracts	Concentration of extract in PPM	% of DPPH free radical Scavenging activity
Aqueous	50	54%
	100	56%
	150	58%
	200	70%
	400	79%
Ethyl acetate	50	42%
	100	55%
	150	59%
	200	64%
	400	66%
Ethanolic extract	50	62%
	100	65%
	150	71%
	200	74%
	400	86%
Dichloro Methane	50	53%
	100	55%
	150	63%
	200	69%
	400	76%

Antioxidant activity of of T. Chebula fruit solvent extracts based on their polarity

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

Extracts	E.coli NCIM 2065	P.aeruginosa NCIM 5029	Klebsiella NCIM 2239	Pseudomonas NCIM 2037	Streptococcus pyogenes NCIM 2608	Staphylococcus aureus NCIM: 2654.
Aqueous	1	2	2	1	2	2
Ethyl acetate	3	2	3	2	4	5
Ethanolic extract	4	3	6	5	7	6
Dichloro Methane	5	3	3	2	1	1

Antibacterial activity of extracts