

In vitro Antibacterial Activity of Bioactive Potent Compounds from *Terminalia chebula* against Some Common Human Pathogens

Shuvo Datta, Nishith Kumar Pal, Ashoke Kumar Nandy*

Department of Clinical & Experimental Pharmacology, School of Tropical Medicine, Kolkata, India

Email: *aknandy_stm@yahoo.in

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Abstract

Objective: Emergence of community-acquired infections due to multi drug resistant (MDR) common human pathogens have caused a great problem to clinicians and this directed us to search systematically for a different remedy with compounds particularly from plant origin. **Methods:** The antibacterial activity was evaluated using agar well diffusion assay method against some common gram positive and gram negative bacteria. **Results:** *In vitro* study with *Terminalia chebula* Retz. (Combretaceae) stem bark extracts, eight isolated triterpenoids and four triterpenoid derivatives were found to be effective against *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Escherichia coli*. **Conclusions:** Pure compounds from *T.chebula* could be effectively used as antibacterial agents if it is possible to develop the molecules synthetically. At the same time crude extracts with specified active principles could also be used and/or introduced in Traditional Medicine/Complementary Alternative Medicine (TM/CAM) as antibacterial into National/International Health Systems as per the guideline of Ayurvedic formularies.

Keywords

Terminalia chebula, Stem Bark Extracts, Triterpenoids, Antibacterial Activity

1. Introduction

Approaches to explore phytochemicals as new therapeutic agents with novel modes of action have already been taken into consideration [1] [2] [3] [4] [5]. Global increase in resistance to antimicrobial compounds, including multidrug resistance among common pathogens, is becoming a critical area of concern to

health care systems in both community and hospital settings and the present scenario demands nothing but the new generation of antibiotics desperately [6] [7] [8]. One helpful clue regarding the search for new antimicrobial compounds is the invincibility of plants against several microbial pathogens [9] [10]. Study suggests that an enormous array of secondary metabolites is produced by the plants and interestingly a significant part of these phytochemicals helps to prevent themselves from pathogens of microbial origin [11]. *Terminalia chebula* Retz. [12] belonging to the family Combretaceae is commonly known as “Haritaki” in both Bengali and Sanskrit. It is called the “King of Medicine” and is always listed first in Ayurvedic Materia Medica for its extraordinary powers of healing with a wide spectrum of biological activity. It has been extensively used in both Ayurvedic and Unani medicines and has become a cynosure of modern medicine. The fruit of this plant is reported to have local application to chronic ulcers and wounds and gargle in stomatitis as well. Finely powdered fruit is used as a dentifrice and considered to be useful in carious teeth, bleeding and ulcerations of the gum. It is also reported to have antioxidant and free radical scavenging activities [13] [14]. Different extracts of the fruits of *T.chebula* were previously found to have antibacterial activity. However, in this study, the *in vitro* effectiveness of stem-bark extracts of *T.chebula* as well as eight isolated triterpenoids and four related derivatives against some common gram-positive and gram-negative bacteria which are generally pathogenic to human beings have been shown.

2. Materials and Methods

2.1. Collection of Plant Material

The stem bark of the plant, *T. chebula* was collected from Bankura district of West Bengal in the middle of Oct’2011 and was authenticated by the Taxonomist, Department of Taxonomy, Shyamadas Baidya Shastrapith, Kolkata, and West Bengal, India. A voucher specimen was deposited in the department of Clinical and Experimental Pharmacology, School of Tropical Medicine, Kolkata.

The freshly collected plant material (stem-bark) was washed with tap water and finally with distilled water. The washed stem-bark samples were shade dried, pulverized mechanically to fine powder and stored in airtight glass containers at 4°C for future use. All experiments were performed with same plant material within nine months from the date of collection.

2.2. Preparation of Extracts

Aqueous extract: For aqueous extraction, 20 g of air-dried, powdered plant material was added to 200 ml double distilled water (DDW) taken in a 500 ml conical flask (graduated) and boiled for 3 h. The volume of the extract was maintained to 200 ml by adding DDW time to time. It was then filtered at room temperature (RT) through cotton wool and centrifuged at 10,000 × g for 15 min. The volume of the collected supernatant was approximately 182 ml. The solution

thus obtained was evaporated under reduced pressure to about 40 ml. This concentrated solution was quantitatively transferred to a 50 ml sterile volumetric flask and finally the volume was made up with sterile DDW. This solution was labeled as T.c.(a) and stored at 4°C to use as water extract.

Methanol extract: 20 g of air dried, powdered plant material was added to 200 ml methanol taken in a 500 ml conical flask. The flask was plugged with cotton and kept on a rotary shaker at 200 - 220 rpm for 24 h at RT. It was then filtered through cotton wool and centrifuged at $10,000 \times g$ for 20 min. The supernatant was then collected and evaporated on an evaporator under reduced pressure to yield a viscous dark greenish brown mass. The above procedure was repeated for extraction in preparative scale. 200 mg of the methanol extract was dissolved in 2 ml pure DMSO (Dimethyl sulphoxide). This solution (concⁿ 100 mg/ml) was labeled as T.c.(m).

Ethanol extract: To prepare ethanol extract, 96% ethanol was used and rest of the procedure was as described as for the methanol extract. DMSO solution of the ethanol extract (100 mg/ml) was labelled as T.c.(e).

2.3. Fractionation/Partition of the Methanol Extract

30 g of the methanol extract was partitioned between 250 ml normal butanol (n-BuOH) and 250 ml DDW. The normal butanol soluble fraction was separated and evaporated on a rotary evaporator under reduced pressure. On complete removal of the solvent, the organic part yielded dark greenish brown mass (9.8 g), 300 mg of this mass was dissolved in 3 ml of pure DMSO and the solution (100 mg/ml) was labeled as T.c.(b). On the other hand 100 ml of water soluble fraction was reduced to approximately 80 ml under vacuum. This solution (~100 mg/ml) was labeled as T.c.(w) and stored at 4°C.

2.4. Isolation of Triterpenoids

7.5 g of n-BuOH extract was chromatographed on silica gel (240 g) and eluted with petrol (40°C - 60°C), Petrol-CHCl₃, CHCl₃, CHCl₃-MeOH (24:1, 19:1, 9:1, 22:3, 17:3, 5:1). Fractions (20 ml each) were monitored by TLC. The CHCl₃-MeOH (19:1) elute (0.6 g) was subjected to prep. TLC using solvent system A (CHCl₃:MeOH:H₂O:80:19:1) to give four chromatographically pure fractions— α (23 mg), β (50 mg), γ (19), δ (21 mg) according to the increasing order of polarity. Fractions— α , β , γ and δ were identified as arjunolic acid (1), arjunenin (2), belleric acid (3) and terminolic acid (4) respectively by comparing (mixed mp, Co-TLC and superimposable IR) with authentic samples [15] [16]. Fractions eluted CHCl₃-MeOH (22:3 and 17:3) were combined (2.3 g) subjected to prep. TLC with solvent system B (CHCl₃:MeOH:H₂O:71:25:4) to afford four chromatographically pure fractions P (142 mg), Q (305 mg), R (138 mg) and S (148 mg) according to the increasing order of polarity. Pure fractions P, Q, R and S were found to be identical as chebuloside I (5), arjunglucoside I (6), bellericoside (7) and chebuloside II (8) respectively by comparing with (mixed m.p, Co-TLC and superimposable IR) with authentic samples [15] [16].

2.5. Biological Testing

Antimicrobial activity: The agar well diffusion assay was used to screen the antimicrobial activities of the stem bark extracts, eight isolated triterpenoids and four triterpenoid derivatives (**Figure 1**). Extracts and pure compounds were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 100 mg/ml and 2 mg/ml respectively.

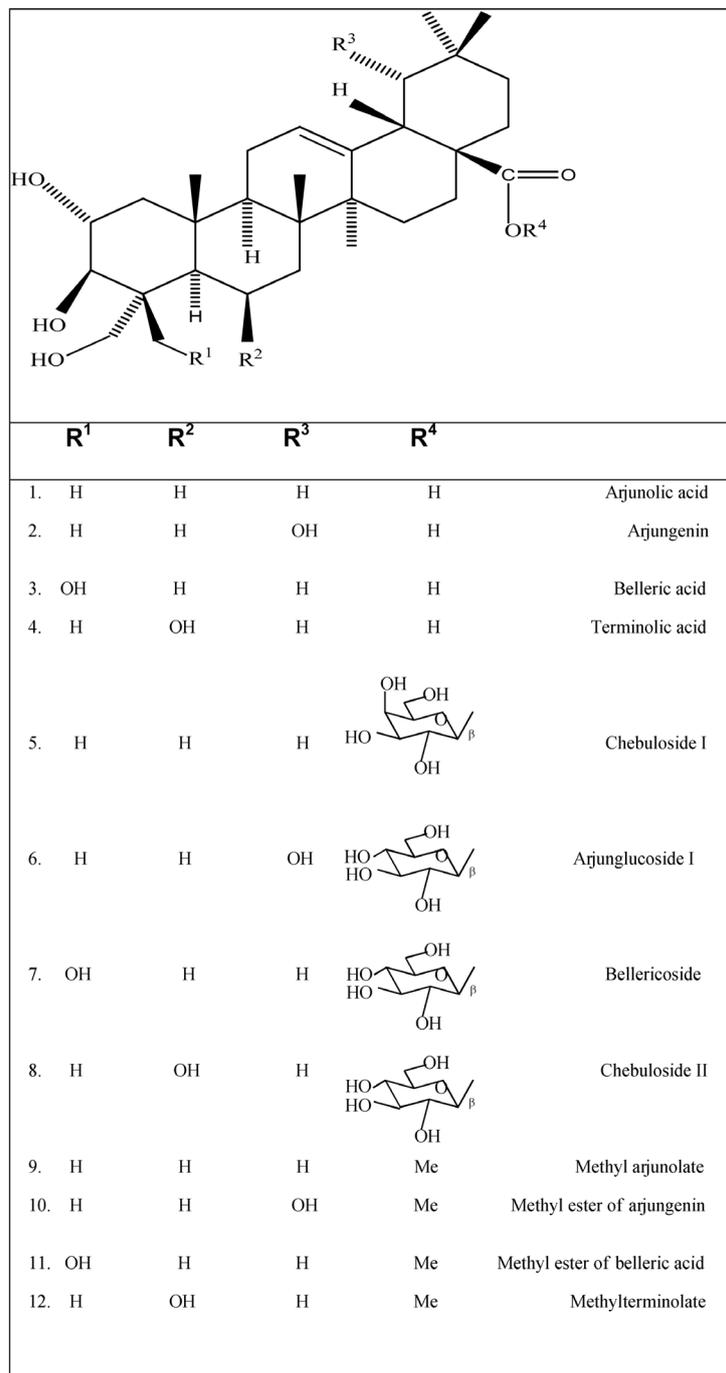


Figure 1. Tested compounds from *T. chebula* R¹, R², R³ and R⁴ indicate the position of the particular atom/group. Me = Methyl group (CH₃).

3. Results

Figure 2 shows the antibacterial activity [17] of aqueous solution [T.c.(a)] and that of DMSO solutions (100 mg/ml) of methanol [T.c.(m)] and ethanol [T.c.(e)] extract as well as that of DMSO solutions (100mg/ml) of water and butanol fractions of the methanol extract [T.c.(w) and T.c.(b)] of the stem-bark of *T. chebula* [18] against some selected bacteria in terms of average inhibition zone diameter (IZD) in mm [19] [20].

The data reported in **Figure 3** shows antibacterial activity of eight naturally occurring triterpenoids, arjunolic acid (1; 2 α , 3 β , 23-trihydroxyolean-12-en-28-oic acid), arjunjenin (2; 2 α , 3 β , 19 α , 23-tetrahydroxyolean-12-en-28-oic acid), belleric acid (3; 2 α , 3 β , 23,24-tetrahydroxyolean-12-en-28-oic acid), terminolic

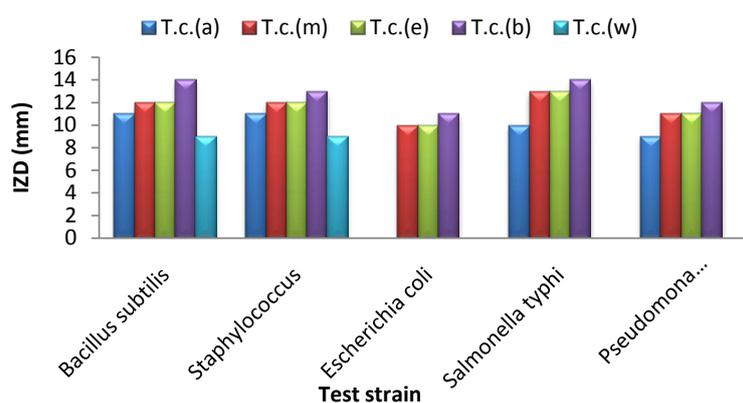


Figure 2. *In vitro* antibacterial activity of different extracts of *T.chebula* stems bark. T.c.(a) = aqueous extract of *T.chebula* (stem-bark); T.c.(m) = DMSO solution of the methanol extract of T.c. (stem-bark) [100 mg/ml]; T.c.(e) = DMSO solution of the ethanol extract of T.c. (stem-bark) [100 mg/ml]; T.c.(b) = DMSO solution of the n-BuOH fraction of MeOH extract of T.c. (stem-bark) [100 mg/ml]; T.c.(w) = Water soluble fraction MeOH extract of T.c. (stem-bark) [100 mg/ml]; Dose: 50 μ l test solutions in each well (6 mm).

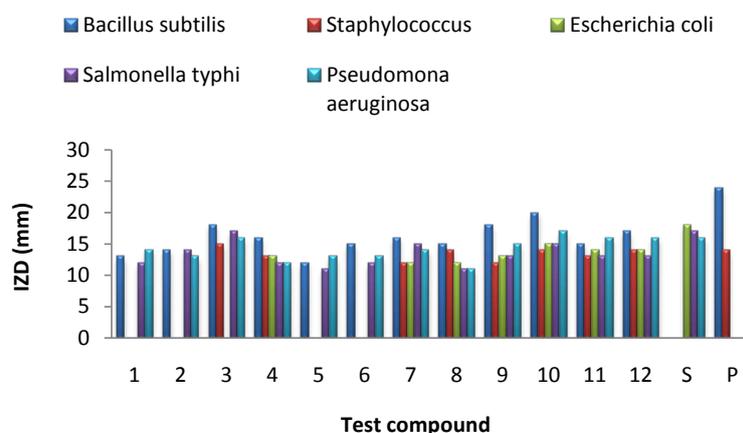


Figure 3. *In vitro* antibacterial activity of twelve triterpenoids (1 - 12) of *T.chebula*. S = Streptomycin sulphate (100 μ g), as a control for gram (-)ve bacteria. P = Penicillin sulphate (1 μ g), as a control for gram (+)ve bacteria, 1 - 12 = Different tested compounds of *Terminalia chebula*, Dose: 50 μ l of 2 mg/ml test solutions in each well (6 mm).

acid (4; 2 α , 3 β , 6 β , 23,-trihydroxyolean-12-en-28-oic acid) and their ester glycosides, chebuloside I (5; β -D-galactopyranosyl 2 α , 3 β , 23-trihydroxyolean-12-en-28-oate), arjunglucoside I (6; β -D-glucopyranosyl 2 α , 3 β , 19 α , 23-tetrahydroxyolean-12-en-28-oate), bellericoside (7; β -D-glucopyranosyl 2 α , 3 β , 19 α , 23,24-tetrahydroxyolean-12-en-28-oate), chebuloside II (8; β -D-glucopyranosyl 2 α , 3 β , 6 β , 23-tetrahydroxyolean-12-en-28-oate) as well as four methyl derivatives of the triterpenoid acids namely methyl arunolate (9; methyl 2 α , 3 β , 23-trihydroxyolean-12-en-28-oate), methyl ester of arjungenin (10; methyl 2 α , 3 β , 19 α , 23-tetrahydroxyolean-12-en-28-oate), methyl ester of belleric acid (11; methyl 2 α , 3 β , 23,24-tetrahydroxyolean-12-en-28-oate), methyl terminolate (12; methyl 2 α , 3 β , 6 β , 23-tetrahydroxyolean-12-en-28-oate) against previously selected bacteria in terms of average IZD in mm. From the **Figure 2**, it is revealed that the aqueous extract [T.c.(a)] had the least [21] activity among the three extracts (aqueous, methanol and ethanol) and obtained by direct percolation of the plant material. Both the methanol and ethanol extracts [T.c.(m) and T.c.(e)] showed almost similar activity. n-BuOH fraction [T.C.(b)] of methanol extract was found to be most effective [22]. On the other hand the water soluble part [T.c.(w)] of MeOH extract unexpectedly showed some efficacy.

Figure 3 unveils the fact that the synthetically prepared derivatives (9 - 12) are somewhat more effective than naturally occurring triterpenoids.

4. Conclusions

In vitro study with *Terminalia chebula* Retz. (Combretaceae) stem bark extracts, eight isolated triterpenoids and four triterpenoid derivatives were found to be effective against some common gram positive and gram negative bacteria isolated from local and patient sources. A comparative study of the efficacy of different extracts of *T.chebula* stem bark was also done. Major constituents of the *T.chebula* fruit are hydrolysable tannins and components thereof, including chebulagic acid, chebulinic acid, chebulanin, corilagin, gallic acid, gallic acid methyl ester, punicalagin, terchebulin and terminolic acid. Flavonols of interest include quercetin, isoquercitrin and rutin [23]. Fruit part of this plant was described to be used orally to treat cough with sore throat, as well as diarrhea in pharmacopoeias and well established documents [24]. In traditional medicine, fruit part was described to use orally as an anti-helminthic, astringent, cardio tonic, dentifrice, diuretic and laxative. It is also used to treat bleeding gums, diabetes, gastrointestinal disorders, ulcers and urinary disorders [25]. In *WHO monographs on selected medicinal plants* [23], it was also mentioned that an aqueous extract of the fruit was active against six dermatophytes, namely *Trichophyton mentagrophytes*, *T. rubrum*, *T. soudanense*, *Candida albicans*, *Torulopsis glabrata* and *C. krusei* *in vitro*. The *in vitro* antibacterial activity of an extract of the crude drug was assessed in the disc diffusion assay. The extract was active (concentration range 30 - 500 μ g/disc) against human pathogenic Gram-positive and Gram-negative bacteria, including *Shigella dysenteriae*, *S. flexneri*,

S. boydii, *Proteus mirabilis*, *P. vulgaris*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella* species. A 50% ethanol extract of the fruit inhibited the growth of methicillin-resistant *Staphylococcus aureus* (MRSA), with a minimum inhibitory concentration of 31.3 µg/ml [26] [27].

However the secondary metabolites of *Terminalia chebula* which were found to be active principle are triterpenoids in nature. Isolable quantity of these triterpenoids were very less, so it is very difficult rather impossible to use these compounds as antibacterial agents unless and until these molecules are prepared synthetically but crude extracts with those active principles could be used and/or introduced in Traditional Medicine/Complementary Alternative Medicine (TM/CAM) as antibacterial into National Health Systems as per the guideline of Ayurvedic formularies. Synthetic approaches for further derivatization of active principles through structure-activity relationship studies may lead to development of new antibiotic(s) of higher potency.

Conflict of Interest Statement

We declare that we have no conflict of interest.

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Drawings: Chemical Structures have been drawn by using ChemBioDraw Ultra.

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References

- [1] Marr, C. and Bent, S. (1999) An Evidence Based Review of the 10 Most Commonly Used Herbs. *Western Journal of Medicine*, **171**, 168-171.
- [2] Cragg, G.M. and Newman, D.J. (2013) Natural Products: A Continuing Source of Novel Drug Leads. *Biochimica et Biophysica Acta*, **1830**, 3670-3695. <https://doi.org/10.1016/j.bbagen.2013.02.008>
- [3] Che, C.T., Wang, Z.J., Chow, M.S. and Lam, C.W. (2013) Herb-Herb Combination for Therapeutic Enhancement and Advancement: Theory, Practice and Future Perspectives. *Molecules*, **18**, 5125-5141. <https://doi.org/10.3390/molecules18055125>
- [4] Khatri, S., Kumar, M., Phougat, N., Chaudhary, R. and Chhillar, A.K. (2016) Perspectives on Phytochemicals as Antibacterial Agents: An Outstanding Contribution to Modern Therapeutics. *Mini-Reviews in Medicinal Chemistry*, **16**, 290-308. <https://doi.org/10.2174/138955751604160201150438>
- [5] Pan, S.Y., Gao, S.H., Zhou, S.F., *et al.* (2012) New Perspectives on Complementary and Alternative Medicine: an Overview and Alternative Therapy. *Alternative Ther-*

- apies in Health and Medicine*, **18**, 20-36.
- [6] Nature (2013) The Antibiotic Alarm. *Nature*, **495**, 141. <https://doi.org/10.1038/495141a>
- [7] Piddock, L.J. (2012) The Crisis of No New Antibiotics—What Is the Way Forward? *The Lancet Infectious Diseases*, **12**, 249-253. [https://doi.org/10.1016/S1473-3099\(11\)70316-4](https://doi.org/10.1016/S1473-3099(11)70316-4)
- [8] Lee, V.C. (2015) The Antibiotic Resistance Crisis: Part 1: Causes and Threats. *Pharmacy and Therapeutics*, **40**, 277.
- [9] Savoia, D. (2012) Plant-Derived Antimicrobial Compounds: Alternatives to Antibiotics. *Future Microbiology*, **7**, 979-990. <https://doi.org/10.2217/fmb.12.68>
- [10] González-Lamothe, R., Mitchell, G., Gattuso, M., Diarra, M.S., Malouin, F. and Bouarab, K. (2009) Plant Antimicrobial Agents and Their Effects on Plant and Human Pathogens. *International Journal of Molecular Sciences*, **10**, 3400-3419. <https://doi.org/10.3390/ijms10083400>
- [11] Dixon, R.A. (2001) Natural Products and Plant Disease Resistance. *Nature*, **411**, 843-847. <https://doi.org/10.1038/35081178>
- [12] Chadha, Y.R. (1976) The Wealth of India. Vol. 10, Council of Scientific and Industrial Research, New Delhi, 164.
- [13] Cheng, H.Y., Lin, T.C., Yu, K.H., Yang, C.M. and Lin, C.C. (2003) Antioxidant and Free Radical Scavenging Activities of *Terminalia chebula*. *Biological and Pharmaceutical Bulletin*, **26**, 1331-1335. <https://doi.org/10.1248/bpb.26.1331>
- [14] Saha, S. and Verma, R.J. (2015) Antioxidant Activity of Polyphenolic Extract of *Terminalia chebula* Retzius Fruits. *Journal of Taibah University for Science*, **10**, 805-812.
- [15] Nandy, A.K., Poddar, G., Sahu, N.P. and Mahato, S.B. (1989) Triterpenoids and Their Glucosides from *Terminalia bellerica*. *Phytochemistry*, **28**, 2769-2772.
- [16] Kundu, A.P. and Mahato, S.B. (1993) Triterpenoids and Their Glycosides from *Terminalia chebula*. *Phytochemistry*, **32**, 999-1002.
- [17] De Boer, H.J., Kool, A., Broberg, A., Mziray, W.R., Hedberg, I. and Lavenfors, J.J. (2005) Antifungal and Antibacterial Activity of Some Herbal Remedies from Tanzania. *Journal of Ethnopharmacology*, **96**, 461-469.
- [18] Chattopadhyay, R.R., Bhattacharyya, S.K., Medda, C., Chanda, S., Datta, S. and Pal, N.K. (2007) Antimicrobial Activity of Black Myrobalan (Fruit of *Terminalia chebula* Retz.) against Uropathogen *Escherichia coli*. *Pharmacognosy Magazine*, **3**, 212-215.
- [19] Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Truck, M. (1966) Antibiotic Susceptibility Testing by a Standardized Single Disk Method. *American Journal of Clinical Pathology*, **45**, 493-496.
- [20] Karou, D., Savadogo, A., Canini, A., Yameogo, S., Montesano, C., Simporé, J., Collizzi, V. and Traore, A.S. (2006) Antibacterial Activity of Alkaloids from *Sida acuta*. *African Journal of Biotechnology*, **5**, 195-200.
- [21] Mirjalili, M.H., Moyano, E., Bonfill, M., Cusido, R.M. and Palazón, J. (2009) Steroidal Lactones from *Withania somnifera*, an Ancient Plant for Novel Medicine. *Molecules*, **14**, 2373-2393. <https://doi.org/10.3390/molecules14072373>
- [22] Chakraborty, S.P., Mahapatra, S.K. and Roy, S. (2011) Biochemical Characters and Antibiotic Susceptibility of *Staphylococcus aureus* Isolates. *Asian Pacific Journal of Tropical Biomedicine*, **1**, 212-216.
- [23] WHO (2009) Monographs on Selected Medicinal Plants. Vol-4, WHO.

- [24] (2005) Pharmacopoeia of the People's Republic of China. Chemical Industry Press, Beijing.
- [25] Farnsworth, N.R. (2005) NAPRALERT Database. University of Illinois at Chicago, Chicago.
- [26] Sato, Y., *et al.* (1997) Extraction and Purification of Effective Antimicrobial Constituents of *Terminalia chebula* Retz. against Methicillin-Resistant *Staphylococcus aureus*. *Biological and Pharmaceutical Bulletin*, **20**, 401-404.
<https://doi.org/10.1248/bpb.20.401>
- [27] Savitha, T., Murugan, K. and Thangamariappan, K. (2013) Antimicrobial Evaluation of *Terminalia chebula* Retz. *IJPBA*, **4**, 268-273.



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