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Research Article

CHARACTERISATION AND IN-VITRO EVALUATION OF TERMINALIA CHEBULA EXTRACT FOR ANTIBACTERIAL POTENTIAL

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ABSTRACT

Objective: The present study investigates the qualitative and quantitative analysis of the major bioactive constitutions of methanol extract of *Terminalia chebula* from the various parts of the Coimbatore district, Tamil nadu, India for their authentication.

Methods: Phytochemical analysis, High-performance liquid chromatography (HPLC), Agar diffusion test, Minimum inhibitory concentration (MIC).

Results: The proximate analysis and phytochemical analysis revealed that *Terminalia chebula* had most of the important phyto-constituents like Alkaloids, proteins, saponins, tannins, flavonoids, phenols, terpenoids, carbohydrates, triterpenoids, thiols, steroids and glycosoides were analyzed qualitatively. High-performance liquid chromatography analysis was confirmed the presence of these components objectively. The Quantitative analysis revealed the presence of saponin, alkaloids, Tannin and flavanoids, which in terms confirms their potential for medicinal use. The *in-vitro* antibacterial test by agar diffusion test was conducted for finished textile samples and it is observed that, the treated textiles material have the potential antibacterial property against wide spectrum of human pathogenic strains like *Staphylococcus aureus (MTCC 737), Escherichia coli (MTCC 1687 pneumoniae (MTCC 6644), Proteus vulgaris (MTCC 742), Salmonella typhi (MTCC 733), B. licheniformis (MTCC 429), M. luteus (ATCC 49732)* and *Pseudomonas Sp.,(MTCC 6628), Corynebacterium Sp (MTCC 8730 and ATCC 3021).* Minimum inhibitory concentration (MIC) values were determined and compared with the positive control (Tetracycline) used.

Conclusion: This study justifies the potential and application of traditional medicine in healthcare sector as an alternative eco friendly way against human pathogenic bacterial strains in wound beds.

Keyword: *Terminalia chebula,* Phytochemical analysis, HPLC, Antimicrobial test, Minimum Inhibition Concentration (MIC).

INTRODUCTION

Recently there are lot of attraction towards natural based herbs as an antimicrobial agent because of its eco friendly and health hazardless nature [1-8]. The traditional Indian systems of Ayurveda and Siddha medicines support the importance of medicinal plants to treat diseases [9].

At the turn of the century, approximately 170 herbal drugs were officially recognized in the U.S.P and N.F. [10] The Director of WHO Traditional Medicine reported in 1993 that 80% of the world population rely chiefly on traditional medicine, mainly plant based, specially for their primary health care needs [11]. In India 70% of populations is reported using traditional medicine for primary health care [12]. The present annual turnover of herbal medicinal products manufactured by large companies is estimated to be approximately US $ 300 million, compared to a turnover of approximately US $ 2.5 billion for modern drugs [13]. *Terminalia chebula* is an important medicinal plant in Indian traditional medicine and it is most frequently used herb in Ayurveda. *Terminalia chebula* is a medium- to large-sized tree distributed throughout tropical and sub-tropical Asia, including China and Tibet. This tree is found in the forests of northern India, Uttar Pradesh and Bengal, and is common in Tamil Nadu, Karnataka and southern Maharastra. *Terminalia chebula* is commonly known as black myroblans in English and harad in Hindi. The *Terminalia* consists of 250 species and widely distributed in tropical areas of the world [14]. The fruit of *Terminalia chebula* is considered as the "king of medicines" by Tibetans and second-to- none by ayurvedic apothecaries, and also held in high regard by other folk medicinal practitioners [15]

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Fig. 1: *Terminalia chebula* fruit, leaf and tree

Terminalia chebula is routinely used as traditional medicine in the name of 'Kadukkaai' by villages of Tamil Nadu in India to cure several ailments such as fever, cough, diarrhea, gastroenteritis, skin diseases, candidiasis, urinary tract infection and wound infections [16]. Extracts from different parts of diverse species of plants like root, flower, leaves, seeds, that exhibit antibacterial properties were applied on cotton material for wound, healthcare care application [17, 18]. It is a well known fact that the demand for the herbal drug treatment of various ailments is increasing and plant drugs from the ayurvedic system are being explored more, not only in India but also globally. As a result, in this study an attempt has been made to analyse the phyto-chemical properties of *Terminalia chebula* by both quantitatively and qualitatively. The HPLC studies performed confirm the presence of active components objectively.

The *in-vitro* antibacterial studies of finished textile material were performed against wide spectrum of wound isolate pathogens and confirmed the potentiality of herb against them. The Minimum inhibitory concentration studies were also conducted to identify the minimum concentration requirement of the drug.

Species identity [19-22].

Taxonomy	**Common names**
Current name: Terminalia chebula,	(Cambodia) : sa mao tchet
Botanical description	(Filipino) : chebulic myrabolan
Kingdom: Plantae	(French) : myrobolan noir
Division: Magnoliophyta	(Lao (Sino-Tibetan)) : somz moox kh'ook
Class: Magnoliopsida	(Malay) : manja puteri (unripe fruits)
Order: Myrtales	(Thai) : samo thai (central)
Family: Combretaceae	(Vietnamese) : chieu lieu xanh
Genus: Terminalia	India : haritaki (Sanskrit and Bengali), harad (Hindi), harada (Marathi and Gujrati) Karkchettu (Telgu) and
Species: chebula	Kadukkaya (Tamil).

MATERIALS AND METHODS

Plant Material Extraction

Terminalia chebula fruits, chosen for this study were sourced from the villages of the Coimbatore District, Tamilnadu, India. The collected quantities of *Terminalia chebula* fruits were shade dried and powdered. 20 gm of powder is dissolved in methanol separately for 24 hours to obtain 20% concentrated solution, resulting in active substances being dissolved. The extract were filtered and used for phytochemical analysis and antimicrobial finishing. The concentration of the extract is 200mg/ml.

Phytochemcial Testing

Qualitative phytochemical analysis of the ethanol extract was performed as follows: Alkaloids with Dragendroff reagent; flavonoids using sodium hydroxide and dilute HCl; phenolic compounds with ferric chloride solution; saponins with ability to produce stable foam; glycosides with Keller-Kiliani test; terpenoids by Salkowski test; steroids with Libermann Burchard reagent and reducing sugars with Fehling's solution test. These phytoconstituents were identified by characteristic colour changes using standard procedure. [23-24]

Quantitative phytochemical analysis

Quantitative Determination of alkaloids, flavonoids [23], saponins [25], tannins [26] were performed as mentioned in the literature.

High Performance Liquid Chromatography (HPLC)

HPLC fingerprints were prepared using waters HPLC equipped with UV-VIS detector. Solvents were pre-filtered and analysis was performed in Symmetry C18 column (4.6 x 250mm). The methanol extracts of *Terminalia chebula* were injected in HPLC system. Injection volume was 20 µl. The flow rate was 0.7ml/min and the active spots were identified.

Application on Textile material

Plain knitted Cotton fabric was desized, scoured and bleached prior to the application of the antimicrobial finish. The methanol extract was applied to the cotton fabric by dipping in the bath with material to liquor ratio of 1:10 and then Pad-dryed. Finally the fabric samples were tested for antimicrobial activity as per the AATCC test standards.

Microorganism

Bacterial cultures used in the present studies were obtained from Microbial Type Culture Collection (MTCC) IMTECH, Chandigarh. The bacterial strains *Staphylococcus aureus (MTCC 737), Escherichia coli (MTCC 1687) Klebsiella pneumoniae (MTCC 6644), Proteus vulgaris (MTCC 742), Salmonella typhi (MTCC 733), B. licheniformis (MTCC 429), M. luteus (ATCC 49732)* and *Pseudomonas Sp., (MTCC 6628), Corynebacterium Sp (MTCC 8730)* were used.

Agar diffusion Method (SN 195920)

The treated and untreated fabric samples were placed in the AATCC bacteriostasis agar (AATCC, 2005), which has been previously inoculated (Mat culture) with a test organism. After incubation, a clear area of uninterrupted growth underneath and along the side of the test material indicates the antibacterial effectiveness of the fabric. The area of the inhibition zone is a measure of antibacterial effectiveness of the material [27].

Determination of minimal inhibitory concentration (MIC)

The minimal inhibitory concentrations (MICs) of the extracts of *Terminalia chebula* against all the test strains were determined by macro broth dilution assay method . Two-fold serial dilutions of all the extracts (6.25 to 200µg/ml) were prepared in tubes with Nytrient Broth (Hi-media, Mumbai, India) as diluents. Each dilution was seeded with 40 µl of test micro-organisms to the standard concentration. Two-fold serial dilution of Tetracycline (6.25 – 200 µg/ml) was used as experimental positive control. The tubes were incubated at 37 C for 24 h. The least concentration of the extract or standard drug showing no visible growth was taken as the MIC [28].

RESULTS AND DISCUSSION

Plants that have biological activities usually contain secondary metabolites which are chemical substances responsible for such activities. Phytochemical screening of the extracts of *terminalia chebulla* fruits showed the presence of the following major secondary metabolites viz., saponins, tannins, steroids, flavonoids and etc

Identification and Quantitative analysis of Phytochemical components

Phytochemical analysis is very useful in the evaluation of some active biological components of plants.

The qualitative and quantitative analysis of Terminalia chebula in Table 1 and 2 represents the phytochemical components in methanol extract of *Terminalia chebula.* Important medicinal phytochemicals such as Tannin, saponins, flavonoids, phenol and glocosides were identified as major components of the *Terminalia chebula*. Quantitative evaluation of extracts showed that tannin content was highest at 10.90%, saponins at 3.32%, flavonoids at 0.68% while10.10% was obtained for phenols as given in Table 2.

Table 1: Phytochemical component analysis

Name of Components	Methanolic Extract of *Terminalia chebula*
Flavonoids	+++
Alkaloids	
Dragondroff reagent	+
Wagners reagent	-
Mayers reagent	-
Tannin	+++
Protein	++
Carbohyrate – Fehlings method	+
Saponin	++
Glycosides	++
Phonols	+++
Thiols	-
Steroids	+
Triterpenoids	+

Table 2: Quantitative analysis of Phytochemical components

Analytical Parameters	Results (%)
Total phenols	10.10
Total flavanoids	0.68
Total tannins	10.90
Total saponin	3.32

HPLC – analysis

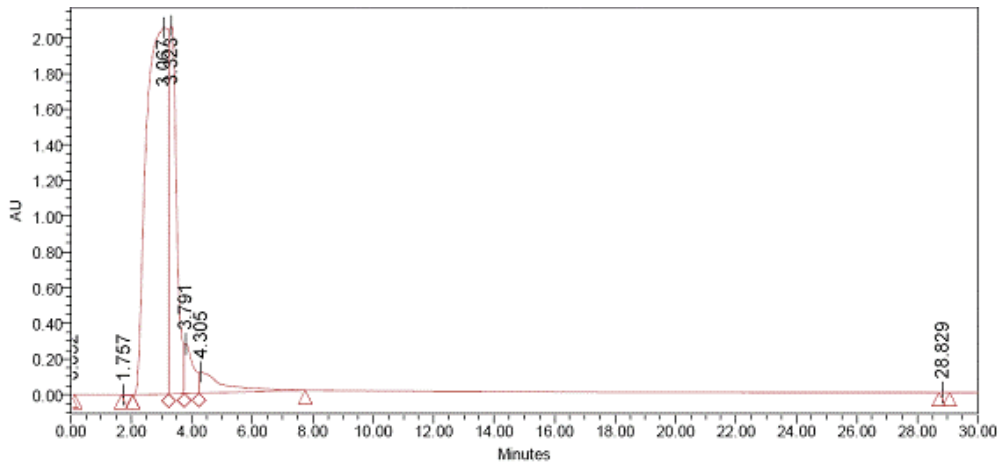

Fig. 2: HPLC Spectrum of *Terminalia chebula*.

Figure 2 shows the HPLC spectrum of crude extract of *Terminalia chebula*. It shows four prominent peaks with the retention time of 3.06, 3.32, 3.79 and 4.3. Out of four peaks the three peaks were compared with standards. The results show that the peaks at this retention times 3.32, 3.79 and 4.3 minutes were identified as Saponin, ascorbic acid and Gallic acid [29].

Anjana Sharma et al [30] also documented the shigellocidal properties of Indian medicinal plants and identified the standard peak for Saponin as 3.31 min. which confirms the presence of Saponin content in the *Terminalia chebula* extract. The presence of gallic acid confirms the phenolic compound is presence objectively.

***In-vitro* antimicrobial Studies**

In vitro antibacterial studies have been carried out for the various human pathogenic bacterial strains based on their presence in human skin and also their presence in various types of wounds. There are several research studies focused on the isolates of wound pus and infection [31-34]. The National nosocomial infections surveillance report represents that, the *Staphylococcus aureus* was recorded as the highest isolation rate of 41% while E. coli recorded the least with 20% presence in the human wound [35]. The study by obi et al identified that, *Klebsiella* spp (25.4%) was the most prevalent organism in the wound samples, followed by *Staphylococcus aureus* with (24.1%); *Pseudomonas* spp was next, (18.2%), then *Escherichia coli*, with 1(18.0%) and finally *Streptococcus pyogenes* with the least percentage (14.1%) [36]. In a similar study elsewhere, Lucinda J Bessa et al [37] isolated 28 species from 217 infected wounds The most common bacterial species detected were *Staphylococcus aureus* (37%), followed by *Pseudomonasaeruginosa* (17%), *Proteusmirabilis* (10%), *Escherichia coli* (6%) and *Coryne bacterium* spp. (5%). It is possible that the type of environment and the state of the wound at any particular time influence the type and prevalent of organisms isolated from a given wound sample. In general, most of the wound isolates having *Pseudomonas aeruginosa* followed by *Staphylococcus aureus, Klebsiella* spp, E. coli, and *Proteus* sp., [38-39].

However, as a consolidated list, *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Proteus vulgaris, Salmonella typhi, Pseudomonas Sp, Bacillus sp, Micrococcus sp* and *Corynebacterium spp* are the most found bacteria in wounds. Based on this survey, to analyse the wound healing ability of the *Terminalia chebula* extracts, the *in-vitro* antibacterial study was carried out for the extract finished textile material by agar diffusion test as mentioned by SN 195920. The result reveals the promising bacterial resistance by the extract of *Terminalia chebula.* Table 3 shows the agar diffusion test zone of inhibition results in mm.

Table 3: Zone of inhibition of treated textile material for different strains

Bacterial strains	Zone of inhibition (in mm)	
	Control	*Terminalia chebula* fruit Methanol extract
Staphylococcus aureus	0	34
Escherichia coli	0	32
Klebsiella pneumonia	0	34
Proteus vulgaris	0	34
Salmonella typhi	0	32
Bacillus sp	0	37
Corynebacterium sp	0	30
Micrococcus sp	0	26
Corynebacterium sp	0	34

The in-vitro antibacterial study of herbal extract finished textile shows potential on the wound healing ability of the selected herb. The ability may be because of the presence of the phytochemical compounds in the extract. Figure 3 shows the strong inhibition of treated cotton textile material against all selected microorganism. The colour change in the agar medium is due to the migration of extract material through the agar. The zone of inhibition should not be expected if the antimicrobial agent is firmly attached to the textile (e.g. covalently) which prevents its diffusion into the agar (in that case the antibacterial activity can be seen only below the fabric sample). If the antimicrobial agent can diffuse into the agar (in case not bonded), a zone of inhibition becomes apparent and its size provides some indication of the potency of the antimicrobial activity or the release rate of the active agent [40].

Among the phytochemical copounds especially alkaloids, saponins, tannins, phenol and flavonoids are known to have curative activity against several pathogens [41] Tannins have been reported to the inhibition of cell protein synthesis as well as production of typical tanning effect which is important in treating inflamed or ulcerated tissues, burns, wounds, pneumonia, and dysentery. Philips [42] reported that tannins and alkaloids are natural products that have medicinal like curing burn wounds to heal injury and cuts to stop bleeding. Moreover, it stop infections on the skin surface, internally tannins continue to heal the wound. In the case of third degree burns using strong tannins sources will not only prevent septicemia, but also helps to save life [43].

Flavonoids have been referred to as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies and virus and they showed their anti-allergic, anti-inflammatory, anti- microbial and anti-cancer activities [44]. The favonoids are potent antioxidants and exhibit various physiological activities including anti-infammatory, antiallergic, anticarcinogenic, antihypertensive, antiarthritic and antimicrobial activities [45] Steroids and triterpenoids showed the analgesic properties. Phenolic compounds are one of the most important groups of compounds occurring in plants [46].

These compounds are reported to exhibit anticarcinogenic, anti-inflammatory, antiatherogenic, antithrombotic, immune modulating and analgesic activities, among others and exert these functions as antioxidants [47].

Saponins are known to have antifungal properties. Saponins have been ascribed a number of pharmacological actions [48, 49]. Saponins are believed to react with the cholesterol rich membranes of cancer cells, thereby limiting their growth and viability [50]. Mudi and Salisu also reported that tannins and saponins exhibit similar antibacterial activities [51]. Therefore, antibacterial activity showed in this present work may be due to tannins, flavonoids and saponins. The wound healing ability of the extract could therefore be attributed to saponins, flavonoids, alkaloids and tannins.

Figure 4 shows the FTIR spectrum of the Terminalia chebula treated and untreated samples. From the spectrum it is identified that the Terminalia chebula treated cotton fabric contain more carboxyl group [C(=O)-OH] due to the presence of active substance like gallic and ascorbic acid in their extract. The absorption in the region 3200 cm-1to 3600 cm-1 and 1200 cm-1to 1700 cm-1 of treated sample confirms the presence of -OH group stretching [52].

The presence of –(C=O)- group stretching also confirms the absorption, in the region of 1600 cm-1 to 1900 cm-1 in the treated sample. This stretching in 1760 cm-1 to 1670 cm-1 confirms the presence of ester group. The Gallic acid could react with cellulosic -OH group which resulted in the ester formation in the treated fabric. The presence of [C (=O)-OH] carboxyl group and – (OH) group confirms the presence of carboxylic acids (ascorbic and Gallic acid) and the presence of ester group proves the deposits of glycosides (Saponin) in the fabric [52].

Minimum inhibitory concentration (MIC) analysis:

In *in-vitro* testing by disk diffusion test mentions the inhibiton as size of the growth-free zone. The MIC number is the lowest concentration (in µg/mL) that inhibits the growth of a given strain of bacteria. The Minimum inhibitory concentration (MIC) studies were performed for the methanol extract of *Terminalia chebula* against all wound isolate test strains.

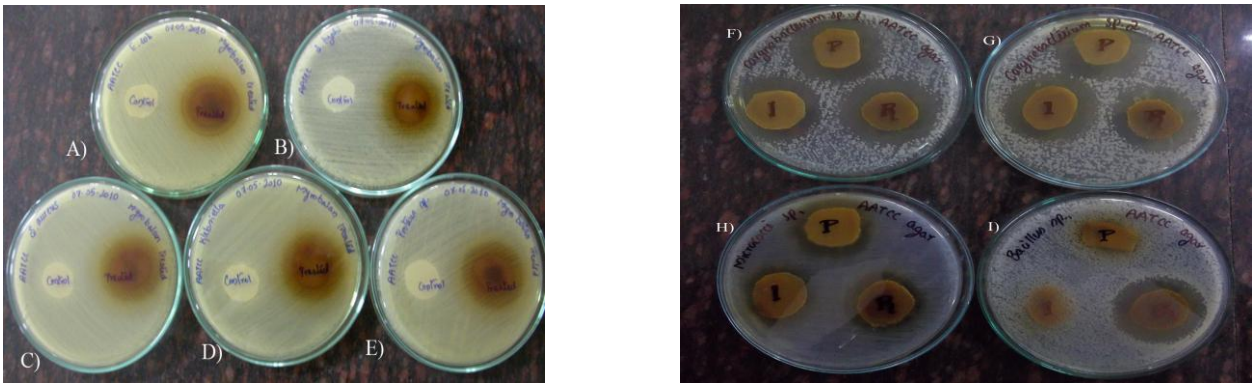

Fig. 3: Agar diffusion test on *Terminalia chebula (mentioned as 'K')* treated cotton fabric: Zone of inhibition against a) *Escherichia coli,* b) *Salmonella typhi*, c) *Staphylococcus aureus*, d) *Klebsiella pneumoniae e*) *Proteus vulgaris f) Corynebacterium sp.,(MTCC 8730) g) Corynebacterium sp., (ATCC 3021) h) M. luteus i) B. licheniformis*

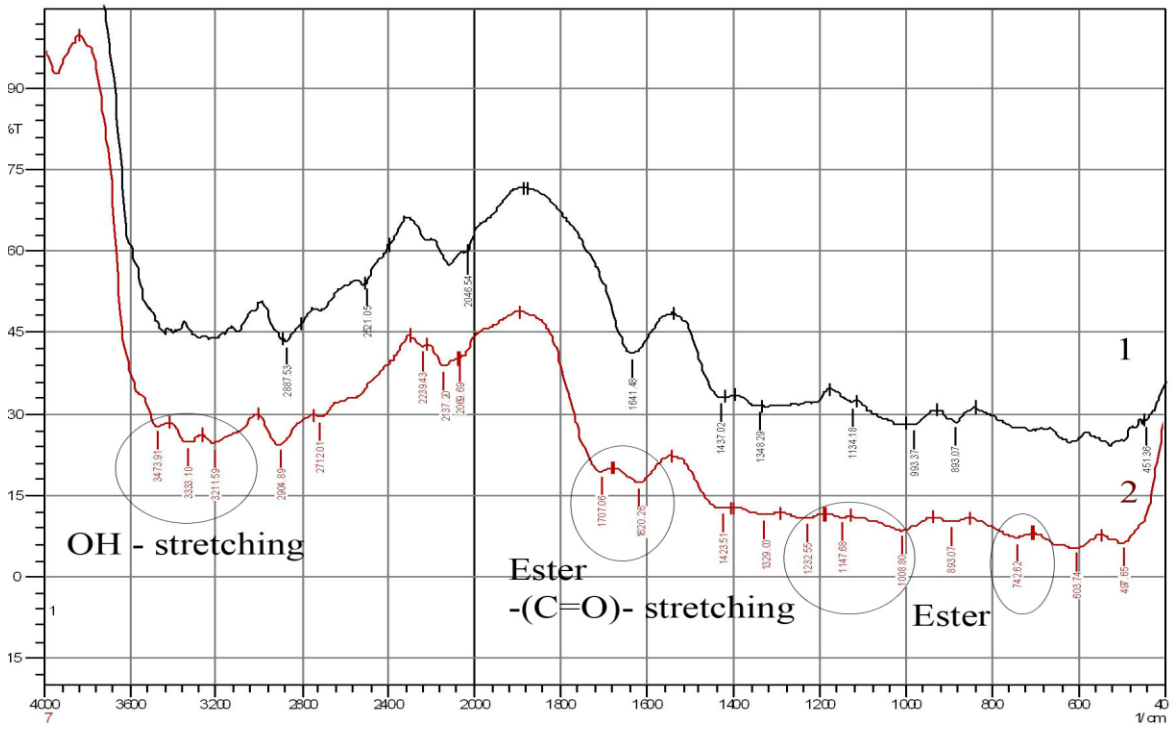

Fig. 4: FTIR spectrum of the *Terminalia chebula* treated and untreated samples.

Table 4: Biological activity of Terminala chebula

Bacterial Strain	1st dilution 200µg/ml	2nd dilution 100µg/ml	3rd dilution 50µg/ml	4th dilution 25µg/ml	5th dilution 12.5µg/ml	6th dilution 6.25µg/ml	Control
Corynebacterium sp.,1	0.02*	0.28	0.49	0.59	0.63	0.72	0.96
Pseudomonas Sp	0.23*	0.29	0.35	0.44	0.72	0.81	
Bacillus licheniformens	0.29*	0.37	0.42	0.49	0.72	0.81	
Micrococci sp.,	0.09	0.09*	0.14	0.19	0.27	0.39	
S.aureus	-0.03	0.17*	0.22	0.29	0.34	0.61	
E.coli	0.22*	0.37	0.42	0.49	0.54	0.61	
Klebsiella pneumonia	0.39*	0.47	0.51	0.75	0.89	0.91	

*Indicates the MIC Point

The study results are shown in Table 4. The result confirms that, the selected herb has the potential effectiveness against all bacterial strains. The results reveals that, minimum of 200µg/ml of *Terminalia chebula* is extract required for the inhibition of *Corynebacterium* sp., In this way, the results identifies that growth of all the test organism were inhibited by the selected extract. Among the tested bacterial wound isolate pathogens, gram-positive bacterial strains have been found to be more susceptible than gram-negative bacterial strains. This may be attributed to the fact that the cell wall in gram-positive bacteria consists of a single layer, whereas, the gram-negative cell wall is a multilayered structure bounded by an outer cell membrane. Thus our findings support the traditional use of *Terminalia chebula* fruits against infections and treated fabric application for wound dressing

CONCLUSIONS

This research helps to spotlight the effectiveness of the traditional medicine kadukkai, which is used in the Indian traditional system for various healthcare purposes. The finding shows that, the herbal treated textile material shows the potential inhibition against the most common wound pathogens in terms of agar diffusion technique. The herb treated textile material shows inhibition up to 37mm of maximum. The Phytochemical analysis shows that, the presence of secondary metabolites viz., saponins, tannins, steroids, flavonoids and etc. These components are the major responsible factor for their antibacterial effect. The quantitative analysis shows that, the phenolic and tannin are the major components. The HPLC and FTIR studies confirmed the presence of phyto components in extracts and fabric respectively. The Minimum inhibition concentration studies were also performed against all test pathogens to measure the effectiveness of the selected herb. This may help in developing an effective alternative antimicrobial agent from plant origin in near future. Further in-vivo studies are needed to uncover the real time relevance of this plant material.

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