Terminalia genus as source of antimicrobial agents

O. Silva* and R. Serrano

RESEARCH INSTITUTE FOR MEDICINES (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Professor Gama Pinto, 1649-003 Lisbon, Portugal

* Corresponding author: email: osilva@campus.ul.pt

Species of Terminalia L. genus (Combretaceae) were worldwide used in traditional medicine to treat different kind of diseases. In Africa, 30 Terminalia species were described and the traditional uses of most of it as antimicrobial agents were reported by different authors. In the present work a summary of the principal studies and results concerning the evaluation of the potential of 11 Terminalia species from Africa, America, Asia and Oceania as antibacterial, antiviral, antifungal and antiprotozoal agents or source of these will be presented. In detail, results obtained by our team concerning the antimicrobial activity of Terminalia macroptera Guill & Perr., a tree widely distributed in the savannas and woodlands of Tropical Africa and used in Guinea-Bissau to treat infectious diarrhoeal diseases and venereal diseases, will be presented and discussed. Extracts of T. macroptera leaf and/or root have been found to be slight active against Candida albicans, and showing an interesting profile of activity against Campylobacter sp., Shigella dysenteriae, Vibrio cholerae, Neisseria gonorrhoeae, Helicobacter pylori and HIV-1. Obtained results corroborate total or partially with the traditional usages of T. macroptera. Also referred are species like Terminalia arjuna, Terminalia bellirica and Terminalia chebula.

Keywords: Antimicrobial; Combretaceae; Ethnopharmacology; Terminalia arjuna; Terminalia bellirica; Terminalia chebula; Terminalia macroptera

1. Introduction

Terminalia L. genus bellows to Combretaceae and consists of trees, often large and sometimes provided with buttresses on the base, and devoid of shrubs scales. Spiral leaves or sub-opposite sometimes grouped in end of the branches, often with glandular petioles and whole limbo or sub entire sometimes gland at the base of the bottom page and, sometimes, with translucent scores. Flowers in spikes or racemes, usually axillary, sessile or pedicellate, in men's rule, to the apex of the inflorescence, and bisexual to the base, mere 5-4-sometimes not merely species Ethiopian), devoid of petals; stamens 10, arranged in two whorls; ovary inferior with 2 (rarely 3) eggs and simple stylus, exert generally pubescent. Very variable fruit, indehiscent, drupaceous or samaroide, to ovoid and roughly angular. Seed with contorted cotyledons [1]. According to Stace, to this pantropical genus have been attributed 400 specific names, corresponding possibly to 100-150 species [1]. Species of this genus were worldwide used in traditional medicine to treat different kind of diseases, including diabetes, inflammatory diseases, malaria and hepatic, venereal and infectious gastrointestinal diseases. Three species of this genus were already considered on British Pharmacopoeia as herbal substances for use in Europe as traditional herbal medicinal products, namely Terminalia chebula Retz. fruit, Terminalia arjuna stem bark and Terminalia bellirica fruit [2]. “Triphala”, an Ayurvedic herbal traditional preparation integrating two of these herbal substances (T. chebula and T. bellirica) among others, is considered the “Ayurvedic wonder” and is traditionally used to the treatment of different types of disease including infectious, since antiquity. The antibacterial activity of these constituents against different strains was already confirmed [3].


Among the studied species: 14 are of African origin, namely Terminalia albida, Terminalia avicennioides, Terminalia brachystemma, Terminalia brownii, Terminalia glaucescens, Terminalia ivorenensis, Terminalia laxiflora, Terminalia macroptera, Terminalia mollis, Terminalia phanerophlebia, Terminalia sericea, Terminalia spinosa, Terminalia stuhlmannii and Terminalia superba; 2 are of American origin, namely Terminalia australis and Terminalia triflora; 6 are of Asian (India) origin, namely Terminalia alata, Terminalia arjuna, Terminalia bellirica, Terminalia chebula, Terminalia nigrovenulosa and Terminalia pallida; and 2 are of Oceanian (Australia) origin, namely Terminalia complanata, and Terminalia muelleri. Although attributed to Asia, the nature range of T. catappa, one of the most important medicinal plants of Terminalia genus, is uncertain. In fact, it has long been naturalized in a broad belt
extending from Africa to northern Australia and New Guinea through Southeast Asia and Micronesia into the Indian Subcontinent. More recently, the plant has also been introduced on America [5]. Polyphenols and triterpenoids are the most common classes of compounds in *Terminalia* genus. Among the polyphenols, flavonoids and hydrolysable tannins (gallotannins and ellagitannins) are predominant, while oleanolic acid derivatives are the major constituents of the triterpene saponins (triterpenoids class of compounds). Besides these classes of compounds, it is also common the presence of high amounts of mucilage and calcium salts (carbonate and oxalate) in this botanical genus (*Terminalia* L.) [6].

The antimicrobial studies on 11 *Terminalia* species, representative of the geographical distribution of this genus in the four Continents and including *Terminalia arjuna*, *Terminalia bellerica* and *Terminalia chebula* were presented and discussed on this work. Obtained data confirm to be the African continent, where 30 species of the genus *Terminalia* are described, the holder of most species of this genus whose antimicrobial activity was determined. During the last 20 years our team has been devoted to the study of *Terminalia macroptera* and it has been chosen as representative species of the African continent, in the present work.

2. Antimicrobial studies on *Terminalia* genus

2.1 African *Terminalia* species

2.1.1 *Terminalia macroptera*


This species is a shrub or small tree 8-10 m tall with a dark-gray rhytidome, deeply fissured and hairless twigs (Fig. 1). This has natural distribution in Western Africa, occurring from Senegal, southward to Nigeria and east to Sudan and Uganda. It is a tree of wooded savannas, usually in situations more or less moist [8].

This species at Guinea-Bissau is known under the name of fadi, fadih (balanta); macêta, macête, macete, masete, masiti (creole of Portuguese matrix) and in French as badamier du Sénégal [9-13].

**Fig 1** *Terminalia macroptera*, general aspect.

Ethnomedical uses: The traditional use of *T. macroptera* is described mostly the countries of West Africa, where it was identified (Fig. 2). For medicinal purposes leaves stems, roots and bark of this species are used [12-14].

The main internal uses, described for *T. macroptera* are the treatment of hepatitis, gonorrhea, syphilis, respiratory tract diseases and fever [15].

Externally, *T. macroptera* is used essentially in treatment of wounds as hemostatic and healing, and treatment of skin diseases [11,16,17]. This species is also used in combination with one or more other medicinal plants belonging to different botanical families [15].

**Fig. 2** West African countries where it is described the use of *Terminalia macroptera*. Traditional herbal preparations: Four main recipes used to administrate *T. macroptera* as medicine, according to pathologies are given:

**Gonorrhea and syphilis:** Remove the rind and cut the root into small pieces, dried in the sun and grind. Boil a portion with water (10 g / L; cooking), add salt and strain with a cloth. Taking a glass until symptoms disappear, once a
day; Guinea-Bissau [18]. Wounds, first apply baking the stem bark, and then, apply a poultice of root bark (hemostatic and wound healing); Guinea-Bissau [11].

**Wounds and conjunctivitis:** Stepping in the bark of roots and pestle in the presence of water, placing the pulp obtained in a clean cloth and squeezing fluid to the wound or on the eye; Senegal [13].

**Jaundice:** Burn the bark of roots, tread the mortar and mix the powder in water; drink the liquid; Togo [15].

**Diabetes, diabetic foot sores:** Leaves and decoction in equal amounts, 1 teacup drunk twice daily for 5–7 days. Mali [18].

Antimicrobial activity studies: The *in vitro* antimicrobial activity of an ethanol extract (95%) obtained by percolation (10 ml ethanol / g dry plant) of leaves, bark and roots of *Terminalia macroptera* against *Bacillus subtilis*, *Staphylococcus aureus* and *Aspergillus niger* was first determined by others. The extract of the leaves showed activity against *Bacillus subtilis* (inhibition zone greater than 15 mm at the concentration of 50 mg/mL) and against *Staphylococcus aureus* (inhibition zone greater than 15 mm at the concentration of 5 mg/mL); The extract of the bark has also shown to be active against *Bacillus subtilis* (inhibition zone less than 15 mm at 50 mg/mL) and against *Staphylococcus aureus* (inhibition zone greater than 15 mm at the concentration of 5 mg/mL); the root extract (50 mg/mL) was shown to be inactive against the 3 microorganisms tested [19].

After a preliminary *in vitro* antimicrobial screening using different reference bacterial strains and *Candida albicans* [20], the antimicrobial activity of the *T. macroptera* roots hydroethanol (80% V/V) extract and liquid-liquid partition fractions was evaluated by our team first against 7 reference bacterial strains *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 43971, *Shigella dysenteriae* ATCC 13313, *Staphylococcus aureus* ATCC 25923, *Streptococcus faecalis* ATCC 10541 and *Vibrio cholerae* ATCC11623, and against *Candida albicans* C1P 3153A. Extract and fractions showed some activity against all strains except *Candida albicans* and *Escherichia coli*. The lowest minimum inhibitory concentrations (MICs) for the extract were obtained with *Shigella dysenteriae* (313 μg/mL), *Staphylococcus aureus* (1250 μg/mL) and *Vibrio cholerae* (less than 156 μg/mL). Similar MICs were obtained with the most active fractions (diethyl ether and water fractions) against the same bacteria. This active extract has been tested against a total of 97 strains of *Escherichia coli*, 106 strains of *Campylobacter* sp., 100 strains of *Salmonella* (*S. enteritis*, *S. typhimurium*) and 100 strains of *Shigella* (*Sh. dysenteriae*, *Sh. flexneri*) isolated from sporadic cases of gastro-enteritis in Portugal, and against 91 strains of *Vibrio cholerae* isolated in Portugal (23 strains), Brazil (38 strains) and Angola (30 strains) Results showed an interesting profile of activity against *Campylobacter* sp. with MIC50 and MIC90 of 25 μg/mL and 50 μg/mL, respectively [21].

Also evaluated was the *in vitro* activity of *T. macroptera* roots hydroethanol (80% V/V) extract and liquid-liquid partition fractions against nine reference and clinical *Neisseria gonorrhoeae* strains, including penicillin- and tetracycline-resistant and susceptible strains. This extract showed MIC values between 100 μg/mL and 200 μg/mL to all tested strains. The most active fractions of this extract were the diethyl ether fraction and water fractions. Ellagic acid, gallic acid, punicalagin, terchebulin, 3,3´-di-O-methylellagic acid, 3,4,3´,4´-tetracylloflavellagic acid and terflavlin A were isolated and identified in the extract and active fractions and also showed activity against *N. gonorrhoeae* strains tested [22, 23].

As referred, *Terminalia macroptera* roots are widely used in African traditional medicine to treat various infectious diseases, including stomach-associated diseases. In a study also conducted by us the *in vitro* activity of the *T. macroptera* roots hydroethanol (80% V/V) extract against 3 standard strains and 62 clinical strains of *Helicobacter pylori* and attempt to localize the extract bioactivity. 20% of the used *H. pylori* strains were clinical isolates resistant to at least two of the three antibiotics belonging to the main groups of antibiotics used in multi-therapy to eradicate *H. pylori* infections. The extract showed anti-*H. pylori* activity against 92% of the tested strains (MIC50 and MIC90 = 200 μg/mL). Water soluble fractions from this extract showed a MIC50 of 100 μg/mL. The main compounds of the extract and active fractions were the ellagitannins terchebulin and punicalagin [24].

In another study an ethanol extract of *T. macroptera* root has shown to be virucidal *in vitro* against a clinical strain of Herpes simplex virus type I (HSV-1) and inactive against a strain of African swine fever virus (ASFV) [25].

Concerning *T. macroptera* leaves the activity of a hydroethanol extract (80%) V/V and liquid-liquid partition fractions were also evaluated by us against 7 reference bacterial strains and against *Candida albicans*. In the range of tested concentrations (3200 to 50 μg/mL), the extract was active against all tested microorganisms. The best results were obtained against *Shigella dysenteriae* and *Vibrio cholerae* (MIC=200μg/mL for both) [26].

In pursuit of our studies, the evaluation of the antimicrobial activity of the hydroethanol extract from the leaves of *T. macroptera* and their Liquid-Liquid fractions against the nine strains *Neisseria gonorrhoeae* used to test the activity of the roots of this species was also perfomed. The results confirmed the antibacterial activity of the leaves extract (MICs between 200 μg/mL and 100 μg/mL) and of the liquid-liquid fractions against all tested strains. Diethyl ether fraction is the most active fraction (MIC between 50 μg/mL and 25 μg/mL). Chebulagic acid, chebulinic acid, isoorientin, gallic acid, ellagic acid and 3,4,5-trimethyl-3’acid, 4’-dioxoloflavellagic acid were among the constituents of the active extract. Gallic acid, ellagic acid and 3,4,5-trimethyl-3’acid, 4’-dioxoloflavellagic acid were simultaneously the main constituents of the most active fraction [27].

A hot aqueous extract from the root bark of *T. macroptera* has shown *in vitro* antiprotozoal activity against *Plasmodium falciparum* W2, a strain resistant to chloroquine (IC50 = 1 mg/mL) [28].
2.2 American *Terminalia* species

2.2.1 *Terminalia australis*

The methanol and aqueous extracts of *T. australis* aerial part showed to be active *in vitro* against to a strain of: *Candida albicans*, with MIC of 180 and 259 μg/mL, respectively; *Candida kruzei* with MIC of 250 and 300 μg/mL, respectively; *Aspergillus fumigatus* with MIC of 0.3 and 0.8 μg/mL, respectively and the methanol extract to *Aspergillus flavus* (1.8 μg/mL). MICs of methanol extract against *Aspergillus* sp. were lower than the MIC of amphotericin B used as positive control. According to the authors, this plant part is not used in traditional medicine [29]. No data correlating the antimicrobial activity with the chemical composition were found.

2.2.2 *Terminalia triflora*

Despite the absence or few reports related with the ethnomedical uses for *Terminalia triflora*, the *in vitro* antimarialar activity of a methanol extract of the aerial parts of this species, from Panama, was evaluated. This extract showed an IC50 value of 9.0 μg/mL against a chloroquine resistant *P. falciparum* strain (W2 Indochina) [30].

At Argentina, results of an *in vitro* antifungal activity study using a methanol extract of this plant part showed its moderate activity against one clinical strain of *Microsporum gypseum*, *Trichophyton rubrum* and *Trichophyton mentagrophytes* (MIC of 250 μg/mL, 100 μg/mL and 100 μg/mL, respectively) [31]. An interesting *in vitro* antifungal activity result was also obtained by Gaitan et al. 2011 [32] using and ethanol extract of the dried leaves of this species. In fact, the extract showed to be active against *Sporothrix schenckii* and *Fonsecaea pedrosoi*, with MIC’s of 25 μg/mL, for both fungi. These microorganisms are usually resistant to most of available antibiotics, and this kind of activity is not commonly studied in medicinal plants [32]. Concerning the antibacterial activity of this species no studies were described. However, and aqueous extract of *T. triflora* leaves showed to inhibit the polymerase and ribonuclease activities of HIV reverse transcriptase at IC50 between 1.6 μg/mL and 1.8 μg/mL, respectively [33] and the ellagitannins punicalin and 2-O-galloylpunicalin were identified as the main compounds responsible for this activity [34].

2.3 Asian *Terminalia* species

2.3.1 *Terminalia alata*

This species is described in the Agroforestry Database [35] as “a medium-sized to fairly large deciduous tree up to 35 m tall, bole up to 200 cm in diameter, bark surface with deep vertical fissures and transverse cracks, dark grey to blackish, inner bark reddish. It is known as “laurel or Indian laurel” and is native from India, Myanmar, Nepal and Thailand.”

Fresh bark juice (3 spoonfuls, 3 times a day for as long as necessary) is taken for diarrhoea and dysentery at Nepal. A methanol extract obtained from dried bark showed to be active *in vitro* against *Staphylococcus aureus*-methicillin sensitive, *Staphylococcus aureus*-methicillin resistant; *Streptococcus faecalis*; *Pseudomonas aeruginosa*-sensitive; *Pseudomonas aeruginosa*-wild type, *Mycobacterium phlei*, *Saccharomyces cervisiae*, *Candida albicans* and *Trichophyton mentagrophytes*. The activity against *S. aureus*-methicillin sensitive was enhanced by UV light, and *S. cervisiae* and *C. albicans* seems to be the less sensitive microorganisms among the tested [37]. However, only one strain of unknown origin of each kind of microorganism was used in this study. Betulic acid, arjunic acid, arjunolic acid, arjunetin and ellagic acid were identified from the trunk bark of this specie [38]. In a ethanol extract of the roots, 3,3’-di-O-methylellagic acid 4-O-β-D-glucopyranosyl-(1--+2)-β-D-glucopyranosyl-(1--+2)-α-L-arabinopyranoside, 5,7,2’-tri-O-methylflavanone, 4’-O-α-L-rhamnopyranosyl-(1--+4)-β-D-glucopyranoside, and 2α,3β,19 β,23-tetrahydroxylean-12-en-28-oic acid 3-O-β-D-galactopyranosyl-(1--+3)-β-D-glucopyranoside-28-O-β-D-glucopyranoside were identified for the first time [39]. No data correlating the antimicrobial activity with the chemical composition were found.

2.3.2 *Terminalia arjuna*

This species, well known as “white marudah“ and considered a sacred medicinal plant, is one of the most important herbal medicines in India and traditionally used as cardio protective and to treat different pathologies including infectious diseases. It is a large tree, up to 6-15 (-25) m tall with smooth, pale greenish to whitish grey bark, distributed throughout the greater part of India, Burma and Sri Lanka [40]. Dried bark is the most used *T. arjuna* plant part and is a cardiac stimulant and cardiotonic herbal medicine. Major constituents responsible for these activities are triterpenoids like arjunolic acid, and arjunic acid, but hydrolysable tannins like arjunin, punicalin, casuarinin, galloylgucose and hexahydroxidephenyl-1-galloyl glucose derivatives, flavon-3-ols such as (+)-catechin, (+)-gallocatechin and (-)-
epigallocatechin and phenolic acids such as gallic acid, ellagic acid and its derivatives like 3-O-methyl-ellagic acid 4-O-beta-D-xylopyranoside and 3-O-methyl ellagic acid 3-O-rhamnoside were also found in Terminalia arjuna [41, 42].

Aqueous and methanol extracts of T. arjuna bark showed to be active against multidrug resistant Escherichia coli DK1 and Staphylococcus aureus MRSA901 tested by agar well diffusion method. The maximum Zone of Inhibition was 9-13 mm for both extracts [43]. However, according to Kan et al. a crude ethanol extract of T. arjuna bark was not active against a clinical multidrug resistant strain of Escherichia coli, Klebsiella pneumoniae and Candida albicans and against ATCC strains of Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis, Streptococcus bovis, Pseudomonas aeruginosa, Salmonella typhimurium, Escherichia coli, Klebsiella pneumoniae and Candida albicans [44]. A methanol extract of this plant and of Triphala (a mixture containing T. chebula, T. arjuna and Emblica officinalis) showed to be active against multi-drug resistant Salmonella typhi strain [45]. Previous studies on this plant part also showed: the antimicrobial activity (1,000–5,000 ppm) of dichloromethane and aqueous extracts against Escherichia coli [46]; and of the crude drug against Bacillus subtilis and Staphylococcus aureus [47].

An anti-Herpes simplex virus effect (HSV) was verified and attributed to the presence of hydrolysable tannins on the bark of T. arjuna [41]. The extract of this medicinal plant is effective at early stage of HSV infection to hinder viral attachment and penetration [48]. The in vitro antiviral activity of casuarinin, one of the hydrolysable tannin isolated from this plant part, was also determined. Results showed that this compound inhibited the viral attachment and penetration, and also disturb the late event(s) of infection [49]. In vitro, T. arjuna bark also strongly suppressed the secretion of hepatitis B virus (HBV) surface antigen (HBsAg) [50]. At Pakistan, the anthelmintic in vitro and in vivo activity of a methanol extract of T. arjuna bark against Haemonchus contortus was also determined. It is active in egg hatch and larval development (645.65 μg/mL and 467.74 μg/mL, respectively) and effective in the adult motility assay (mortality of H. contortus at different hours post exposure). In sheep treated with this extract (3 g/kg) a maximum (87.3%) egg count percent reduction was obtained on day 11 post-treatment [51].

Methanol and aqueous extracts of T. arjuna leaves also showed antihelmintic activity in vitro against Trichostrongylus colubriformis, with a lethal concentration 50 (LC50%) of 1.502 and 3.002 , respectively [52].

### 2.3.3 Terminalia bellirica

A native species on Bangladesh, Bhutan, Cambodia, China, Indonesia, Laos, Malaysia, Nepal, Pakistan, Sri Lanka, Thailand, Vietnam, and according to Orwa et al. 2009 it “is a large deciduous tree to 50 m tall and a diameter of 3 m with a rounded crown. The frequently buttressed bole at the base is branchless up to 20 m. The bark is bluish or ashy-covered with numerous fine longitudinal cracks, the inner bark yellowish” [35]. The fruit rind (pericarp) is used in traditional medicine to treat different diseases including infections and inflammations [35].

As referred, this medicinal plant is commonly used in combination with the fruits of Terminalia chebula and Emblica officinalis is (equal parts of each) in a traditional Ayurvedic herbal preparation called Triphala used to treat different diseases, including gastrointestinal disorders, since antiquity. This preparation has been object of different studies concerning the evaluation of its antimicrobial potential [53]. Aqueous extracts of these plants were prepared and antibacterial activities were evaluated by agar well diffusion method against Escherichia coli and Staphylococcus aureus [54]. Ethanol and aqueous extracts of each component of this preparation and also the total traditional herbal preparation revealed to be active against most of the tested clinical strains of Pseudomonas aeruginosa, Klebsiella pneumoniae, Shigella sonnei, Shigella flexneri, Staphylococcus aureus, Vibrio cholerae, Salmonella paratyphi-B, Escherichia coli, Enterococcus faecalis, Salmonella typhi) isolated from HIV infected patients [55]. The same kind of extracts of each of these three species and of Triphala were also tested against strains of Staphylococcus aureus and Helicobacter pylori isolated from different HIV infected patients. These samples showed and minimal inhibitory concentration between 0.1 and 100 μg/mL to the different tested strains [56]. Other study concerning and ethanol extract of Triphala and the ointment of this extract (10% w/w) revealed in vivo the antibacterial and wound healing capacity of this preparation and its usefulness in the management of infected wounds [57]. Methanol and aqueous extracts of Triphala also showed antibacterial activity against multi-drug resistant Salmonella typhi wounds [58].

Interesting antimicrobial results were also obtained with T. bellirica in other studies. In one of them, made with different medicinal plants used in Thai traditional medicine, the anti-quorum sensing activity a bio monitor strain, Chromobacterium violaceum DMST 21761 was evaluated. T. bellirica was one of the active medicines showing a significant inhibitory activity [59]. In other, the antibacterial activity of T. bellirica fruits against 4 clinical strains of methicillin resistant Staphylococcus aureus (MRSA) and a strain of methicillin sensitive S. aureus (MSSA) isolated from diseased human eyes was determined and these medicinal plants showed broad-spectrum antibacterial activity against all MRSA and the MSSA strains with inhibition zone size of 17–27 mm and a minimum inhibitory concentrations between less than 1500 μg/mL to 8200 μg/mL [60].

The vibriocidal activity against 12 isolates of Vibrio cholerae non-O1, and one reference strain of each Vibrio cholerae and Vibrio parahaemolyticus was also determined on Terminalia bellirica fruits aqueous, acetone, and ethanol extracts. Ethanol extracts showed a MIC between 2500-20000 μg/mL [61]. A T. bellirica fruits ethanol extract showed also some activity against Candida albicans (MIC of 7000 μg/mL) A T. bellirica fruit ethanol extract was tested in vitro using growth inhibition assay for resistance modifying agents (RMAs) of novobiocin against Acinetobacter baumannii,
an important nosocomial pathogen. At 250 μg/mL the extract showed a low antibacterial activity but significantly enhanced the activity of novobiocin at 1 μg/mL against this pathogen [62].

### 2.3.4 Terminalia chebula

This species is a medium size to large tree, up to 25-30 cm tall with many spreading branches with pale greenish, gray and smooth bark, expontanea on India, Ceylon, Burma, Malayan peninsula, Siam an cultivated in Pakistan. In English is known as black myrobalan or chebulic myrobalan. Two varieties of this species are recognized - *Terminalia chebula* var. *chebula* and *Terminalia chebula* var. *tomentella* [40]. It is called the “king of medicines” and is the most important medicinal plant in the Ayurvedic materia medica due to its general use. The fruit of this species is one of the constituents of Triphala, a popular traditional herbal preparation used also for different chronic diseases like diabetes [63].

The dried ripe fruit of *T. chebula* is the plant part traditional used in Indian traditional medicine as homeostatic, antitussive, laxative, diuretic and cardiotonic agent, and to treat chronic ulcers and wounds. The principal class of compounds present on this herbal medicine are triterpenoids (oleanolic acid derivatives) and hydrolysable tannins, like tannic acid, as defined strutures like chebulic acid, chebulagic acid, chebulinic acid, corilagin, punicalagin, chebulanin, corilagin, neochromebulinic acid, 1,2,3,4,6-penta-O-galloyl-H-D-glucose, 1,6-di-O-galloyl-D-glucose, casuarinin, 3,4,6-tri-O-galloyl-D-glucose and terebenein and phenol acids like gallic acid and ellagic acid [63].

*T. chebula* fruit ethanol and water extracts showed to be active against *Bacillus subtilis* ATCC 6051, *Salmonella typhimurium* ATCC 23564, *Pseudomonas aeruginosa* ATCC 25619, and *Escherichia coli* K-12 and *Staphylococcus aureus* clinical strains. The water extract was also active against *Proteus vulgaris* ATCC 6380 using a modified agar well diffusion method. At a concentration of 200 mg/mL this extract was disproved of cellular toxicity (fresh sheep erythrocytes) [64].

Results from another antibacterial activity study made with an ethanol extract of *T. chebula* fruits showed the activity of this herbal medicine against both gram-positive and gram-negative bacteria. This extract was effective against *Salmonella typhi* SSFP 4S, *Staphylococcus epidermidis* MTCC 3615, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* MTCC 441 and *Pseudomonas aeruginosa* ATCC 27853 [65]. Gallic acid and its ethyl gallate were identified as 2 of the main constituents responsible for the antibacterial activity of *T. chebula* fruit against mecthillin-resistant *Staphylococcus aureus* [66].

An aqueous extract and powdered *T. chebula* fruits showed to be active against ten clinical isolates of *Helicobacter pylori* - MICs of 125 μg/mL and 150 μg/mL, respectively and minimum bactericidal concentration of 150 μg/mL and 175 mg/L, respectively. The aqueous extract (1-2.5 mg/mL) also inhibited urease activity of *H. pylori* [67]. This kind of extract also inhibited the growth, sucrose-induced adherence and glucon-induced aggregation of *Streptococcus mutans* [68]. The aqueous extract of black myrobalan also showed antifungal activity against dermatophytes and *Candida albicans* [69].

*In vitro* and *in vivo* antiviral activity of this medicinal plant against HSV and human cytomagalogovirus (CMV) was also demonstrated. This medicinal plant is active against human immunodeficiency virus-1 reverse transcriptase [70] and from this gallic acid and derivatives were isolated as immunodeficiency virus type 1 (HIV-1) integrase inhibitors [71] and other hydrolysable tannins as hepatitis C inhibitors [72]. In combination with acyclovir *T. chebula* showed a stronger *in vitro* antiviral activity against herpes simplex virus type 1 [73].

### 2.3.5 Terminalia nigrovenulosa

This species is a tree or shrub to 15 m tall with a trunk to 0.5 m dbh (diameter at breast height) and a bark gray-white, gray, yellowish brown, gray-brown, or brown, spotted. It is described on Hainan [Cambodia, Laos, Malaysia (NW Peninsula Malaysia and Lankawi Islands), Myanmar, Thailand, Vietnam] [40].

A methanol extract of *T. nigrovenulosa* bark tested by disk diffusion method, showed *in vitro* antifungal activity against the phytopathogens *Phytophthora capsici* KACC 40157, *Fusarium solani* KACC 40384, *Fusarium oxysporum* KACC 40032 and *Rhizoctonia solani* KACC 40111. The methanol leaves extract is also tested and showed to be inactive against *P. capsici* and less active against *R. solani* [74].

### 2.3.6 Terminalia pallida

This species is a semi-evergreen tree recorded from the States of Andhra Pradesh and Tamil Nadu in India and well known as “white gali nut” tree. It is harvested for its fruits and it has been estimated that 40% of the wild population had declined over three generations (87 years). Therefore this species is actually referred by IUCN on The IUCN Red List of Threatened Species and assessed as Vulnerable by this organization [75].

Traditionally, the bark is used has mild diuretic and the fruits are used in the treatment of ulcers, diarrhea and in venereal diseases. An *in vitro* antimicrobial screening using a methanol extract of fruit powder of this species at a concentration range of 625-5000 μg/mL by cup-plate method showed that this extract exhibited activity against one
clinical strain of each Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus luteus, Escherichia coli, Salmonella typhi, Shigella dysenteriae, Vibrio cholerae, Aspergillus niger and Candida albicans [76].

2.4 Oceanian Terminalia species

2.4.1 Terminalia complanata

A rainforest species described on Australian Flora as a “tree to 45 m, deciduous or partly so. Branchlets sericeous, glabrescent”, occurring in Australia from Iron Ra. and McIlwraith Ra., Qld, but extending through New Guinea to the Solomon Islands [77].

No data related with medicinal traditional uses of this species were found in the literature. T. complanata leaf, root and stem bark methanol extracts were tested against bacteria, protozoan and fungi (Aspergillus niger, A. rubrum, A. versicolor, A. viti, Candida albicans, C. tropicalis, Cladosporium cladosporiodes, Trychophyton mentagrophytes, T. tronsum) by disk diffusion method (20 mg/disc). The extracts were inactive against all tested moulds but active against all other microorganisms tested (Bacillus cereus, B. coagulans, B. megatarius, B. subtilis, Lactobacillus casei, Micrococcus luteus, M. roseus, Staphylococcus albus, S. aureus, S. epidermidis, Streptococcus faecalis, St. mutans, St. pneumonia, Agrobacterium tumefaciens, Citrobacter freundii, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumonia, Neisseria gonorrhoeae, Proteus mirabilis, P. vulgaris, Pseudomonas aeruginosa, Salmonella typhi, S. typhymurium, Serratia marcescens and Trichomonas vaginalis) [78]. Arjunaolic acid and asiatic acid were found in T. complanata wood [79].

2.4.2 Terminalia muelleri

This species is described on ABRS Flora of Australia (2015) as a “tree to 10 m, deciduous. Branchlets appressed pubescent, glabrescent. Occur from Rockhampton to Cape York, Qld, extending a small distance S along the western side of Cape York Peninsula. Common in low closed-forest on stabilised dunes and occasionally on sandstone on the coast.” [80].

This plant is known in Indonesia as “ketapang kencana”. The ethanol extract of the leaves has shown antimicrobial activity against Escherichia coli, Staphylococcus aureus and Candida albicans. Additionally, non-identified constituent of ethylacetate extract of this plant part has shown inhibit a methicillin-resistant Staphylococcus aureus strain [81-83].

3. Final remarks

The studies described clearly show the interest of the botanical Terminalia genus as source of medicines with antimicrobial activity. Most studies were performed in vitro and focus primarily the determination of the antibacterial activity of the target species. In fact, few antiviral and antimalarial activity studies were made until now on this botanical genus.

The majority of the antimicrobial activity studies were conducted in non-standardized extracts and even of unknown composition. In addition the constituents responsible for the antimicrobial activity were not determined. Thus, taking into account the possible variability in the vegetable starting material and the different methods used for the production of the plant extracts tested it may be justifiable the variability between the results obtained in different studies involving the same medicinal plants and the same microorganisms. Hydrolysable tannins is one of the classes of chemical compounds that can be correlated with the demonstrated antimicrobial activity demonstrated by the different Terminalia medicinal plants.

Studies made with Terminalia macroptera decorticated root showed antimicrobial activity relevant against Neisseria gonorrhoeae, consistent with the traditional use in the treatment of venereal diseases. Additionally, this medicinal plant was shown to be active against Staphylococcus aureus and Enterococcus faecalis, which is consistent with the traditional use for the treatment of conjunctivitis and wounds, and urinary problems in other West African countries. This part of the plant showed large activity against bacteria commonly associated with the onset of diarrhea (Salmonella typhimurium, Shigella dysenteriae) and cholera (Vibrio cholerae), infectious diseases of high incidence in Africa. In addition, this herbal medicine proved to have virucidal activity against HSV-1. The major constituents of the active herbal preparation were identified and correlated with the biological activity. The same correlation was verified on Terminalia macroptera leaves studies.

Acknowledgements  The support by iMed.ULisboa (UID/DTP/04138/2013) from Fundação para a Ciência e a Tecnologia (FCT), Portugal, is gratefully acknowledged.
References


[17] Pobéguin H. Les Plantes Médicinales de la Guiné, Paris, A. Challame; 1912; p.27


[40] *Terminalia arjuna; Terminalia chebula; Terminalia nigrovulnerosa*. In Flora of Pakistan @ efloras.org [cited 2015 Jun 29]. Available from: http://www.efloras.org


