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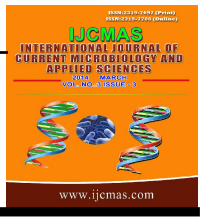

Original Research Article

Phytochemical analysis and antibacterial activity of extracts from *Terminalia chebula* Retz

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A B S T R A C T

The present study deals with the fruit extracts of *Terminalia chebula* Retz. contained different types of phytochemicals such as glycosides, alkaloids, flavonoids, phenolic compounds, saponin, steroids, quinine and tannin. The antibacterial activity of crude extract of *T. chebula* Retz. was studied against gram-negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* and gram-positive bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*. The antibacterial activity was studied by disc diffusion method. Extracts with the different solvent of *T. chebula* Retz. exhibited the antibacterial activity bacterial strains. In general, all extracts inhibited the growth of all test microorganisms and in disc method, with the range of concentration of 100µl, 150µl and 200µl the extract, the growth of all microorganisms was inhibited and also showed dose – dependent activity. Of the eleven solvent used methanol, ethanol and acetone seems to be the best solvent when compare to other solvents.

Keywords

Terminalia chebula Retz;, Phyto-chemical analysis; Antibacterial Activity.

Introduction

Medicinal plants are the plants or their parts used for the health care. They probably constitute a single larger functional group of the plants globally. According to an estimate, 120 or so plant based drugs prescribed for use through the world come from just 95 plant species (Lewington, 1990).The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body (Akinmoladun *et al.*, 2007). Some of the most important bioactive phytochemical constituents are alkaloids, essential oils, flavonoids, tannins, terpenoid, saponins, phenolic compounds and many more (Edeoga *et al*., 2005). These natural compounds formed the foundations of modern prescription drugs as we know today (Goh *et al*., 1995). Phytochemical is a natural bioactive compound found in plants, such as vegetables, fruits, medicinal plants, flowers, leaves and roots that work with

nutrients and fibers to act as an defense system against disease or more accurately, to protect against disease. Phytochemicals are divided into two groups, which are primary and secondary constituents; according to their functions in plant metabolism. Primary constituents comprise common sugars, amino acids, proteins and chlorophyll while seconddary constituents consists of alkaloids, terpenoids and phenolic compounds (Krishnaiah *et al*., 2007).

When blood circulation gets impaired and dropsy sets in, *Digitalis* helps in restoration and regulation of the function of the heart. It improves the blood supply to the kidney and this promotes urination and removes obstructions in kidney. The antibacterial activity of leaf extracts were tested against each multi resistant bacterium by disc diffusion method (Berghe and Vlietinck, 1991). Despite the existence of potent antibiotic and antifungal agents, resistant or multi-resistant strains are continuously appearing, imposing the need for a permanent search and development of new drugs (Silver, 1993). It is therefore very necessary that the search for newer antibiotic sources be a continuous process. Plants are the cheapest and safer alternative sources of antimicrobials (Pretorius and Watt, 2001). *Moringa oleifera*. is the most widely cultivated species of a monogeneric family, the Moringaceae that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan (Fahey, 2005) which is widely used for treating bacterial infection, fungal infection, anti-inflammation, sexually-transmitted diseases, malnutrition and diarrhoea. *Moringa* species have long been recognized by folk medicine practitioners as having value in the treatment of tumors (Ramachandran *et al*., 1980).

Materials and Methods

Plant materials

Plant materials (Fruits of *Terminalia chebula* Retz.) were collected from the location of Rajapalayam, Virudhunagar District. The plant materials were identified in the Department of Botany, Ayya Nadar Janaki Ammal College, Sivakasi.

Microorganism used

Gram-negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* and gram-positive bacteria such as *Bacillus subtilis*, *Staphylococcus aureus* were obtained from the Department of Microbiology, Ayya Nadar Janaki Ammal College, Sivakasi, India.

Preparation of plant extract

Fruit extracts were prepared according to the method described by (Ahmad *et al.* 1998) with minor modification. The fruit extract thoroughly washed with distilled water and then dried under shade condition. The dried fruit were powdered and stored in air sealed plastic container at room temperature until the time of extraction. The fruit powders were subjected to extraction using organic solvents. 5g of powered plant material was soaked in 10ml of solvent for 72 hours, with stirring every 24 hours. At the end of extraction period, it was centrifuged and supernatant was filtered through Whatman No.1 paper. This extraction was repeated three times. Filtrates were pooled and evaporated to air dry and stored at 20^0C for further use.

Solvent used

The solvent such as methanol, ethanol, acetone, ethyl acetate, hexane, chloroform, petroleum ether, n-butanol, n-propyl alcohol, aqueous and diethyl ether were used for extraction purpose.

Phytochemical analysis

Phytochemical analysis for major phytoconstituents of the plant extracts undertaken using standard qualitative methods as described by various authors (Vogel, 1958; Kapoor et al., 1969; Fadeyi *et al.*, 1989; Odebiyi and Sofowora, 1990). The plant extracts were screened for the presence of biologically active compounds like glycosides, alkaloids, flavonoids, phenolic compounds, saponin, steroids, quinine and tannin (Harborne, 1973). Chemical tests are conducted on the aqueous extract of each plant sample and also of the powdered form of the plant samples using standard methods (Edeoga *et al*., 2005).

Test for Alkaloids: Dragendroffs reagents

Solution A: 0.6g of Bismuth sulphate dissolved in 20ml of water.
Solution B: 6g of Potassium iodide was dissolved in 50ml of water.
Solution A and Solution B were mixed and allowed to strand for some time. The supernatant was decanted from potassium iodide and make up to 100ml.

Test for Flavonoids: 1ml of stock alcoholic solution with few drops of neutral $FeCl_3$ and 5ml of extract with 1ml of alcohol subjected to the Ferric chloride test.

Test for Phenolic compounds: 1ml of extract with 5ml of alcohol and few drops of neutral $FeCl_3$.

Test for Tannin: 1ml of extract with minimum amount of H20. Filtered and to the filtrate add few drops of $FeCl_3$ solution.

Test for Saponins: 1ml of extract with 20ml of distilled water agitated vigorously for 15 minutes.

Test for Steroids: 1ml of extract with 1ml of methanolic extract of drug and 1ml of chloroform, 2 -3ml of acetic anhydride and 1 -2 drops of conc. H_2SO_4 were added.

Test for Quinine: 1ml of extract with few drops of alcoholic KOH was added.

Test for Glycosides: 1g powder with dissolved in 2 -3 ml of distilled water and 2 -3 drops of 1per cent solution of alcoholic -napthol added side of test tube.

Antibacterial activity

The antibacterial test was carried out against gram positive and gram negative bacteria. The antibacterial activity of leaf and fruit extracts were tested against bacteria by disc diffusion method (Berghe and Vlietinck, 1991; Cappouccino and Sherman, 1998). 100µl, 150µl and 200µl concentration extract loaded disc were placed on the surface of the agar medium by pressing with sterile forceps in an aseptic condition. The inoculated and treated plates were incubated at 37^0C for 24 hours. After the incubation, the diameter of zone was measured. Standard antibiotic streptomycin (50mg/ml) used as control. The respective control was also run simultaneously using different solvents to compare the effect of plant extracts. After overnight incubation, the

diameter of each zone of inhibition was measured. In all measurements, the zones of inhibition are measured from the edges of the last visible colony-forming growth. The results are recorded in millimeters (mm) and interpretation of susceptibility is obtained by comparing the results to the standard zone sizes.

Results and Discussion

Results obtained from premilinary phytochemical and antibacterial activity of fruit extract of *Terminalia chebula* Retz. The present study indicated that the *T. chebula* Retz. contained different types of phytochemicals such as alkaloids, flavonoids, saponin, phenolic compounds, steroids, carboxylic acid, tannin and glycoside. Presence of phytochemicals differed in different types solvent in the fruit extracts. Similar result was obtained by different researchers in different plants. Alkaloids reacted with Dragendroffs reagents to produce reddish brown precipitate, indicate the presence of alkaloids in the fruit extracts (Table 1). Ferric chloride reacted with fruit extract and formed blue colour precipitate. The above result indicated the presence of tannins in the extract of *T. chebula* Retz. (Table 1) Glycosides reacted with α-napthol and sulpuric acid to form brick red colour. (Table 1) Steroids reacted chloroform, acetic anhydride and concentrated sulphuric acid to produce rosy red colour, indicated the presence of steroids. Saponin reacted with mercuric chloride to produce white precipitate as positive result (Table1)**.** Flavonoids reacted with ferric chloride to form blackish red colour confirm the presence of flavonoids (Table 1)**.** Quinine reacted with alcoholic NaOH solution to colour change from red to blue (Table 1). Phenolic compounds reacted with neutral $FeCl_3$ to colour change as positive result (Table 1).

The phytochemical analysis of the *P. guajava* extract revealed the presence of tannins while that of *M. indica* showed the presence of alkaloids, saponins and tannins. These compounds are known to be biologically active. Tannins have been found to form irreversible complexes with proline-rich proteins (Hagerman and Butler, 1981) resulting in the inhibition of the cell protein synthesis. This activity was exhibited against test organisms with the two plant extracts. Tannins are polyphenols that are obtained from various parts of different plants (Gajendiran and Mahadevan, 1990). In addition to use in leather processing industries, tannins have shown potential antiviral (Lin *et al.*, 2004), antibacterial (Akiyama *et al.*., 2001), and antiparasitic effects (Bhagavathi *et al*, 1999; Yang *et al.*, 1999). In the past few years tannins have also been studied for their effects against cancer through different mechanisms.

Methanol extract of *T. chebula* Retz. inhibited the growth of bacteria and gave inhibition zone range of 11-17mm at 200μL concentration (Fig 2). Similar result was obtained with ethanol extract (Fig 3) Methanol extract of *T. chebula* Retz. produced more inhibition zone with *K. pneumonia* (17mm) than others. There was not much variation in inhibition zone with acetone (Fig 4), n-propyl alcohol and ethyl acetate extracts in all concentration against all tested bacteria (Table 2 and 3). Comparatively, hexane extract was more effective against *E. coli* and less effective with *P. aeruginosa*. Petroleum ether extract of *T. chebula* Retz showed no activity against *B. subtilis, S. aureus, P. aeruginosa* and *K. pneumoniae*, while inhibited growth of *E. coli* in all

Table.1 Phytochemical analysis in *Terminalia chebula* Retz with different solvents

S.No	Extract	Phytochemicals							
		A	F	S	S	Q	PC	T	G
1.	Acetone	+	+	-	-	-	+	+	-
2.	Ethyl acetate	+	+	-	-	-	+	+	-
3.	Hexane	+	+	-	-	-	+	+	-
4.	Ethanol	+	+	-	-	-	+	+	+
5.	petroleum ether	+	+	-	-	-	+	+	+
6.	Chloroform	+	+	-	-	-	+	+	+
7.	Diethyl ether	-	+	-	-	+	+	+	+
8.	n-propyl alcohol	+	+	-	-	-	+	+	+
9.	n-butanol	+	+	-	-	-	+	+	+
10.	Methanol	+	+	-	-	+	+	+	+
11.	Water	+	+	-	-	-	+	+	+

A – Alkaloid, PC - Phenolic compound, F – Flavonoid, T - Tannin S – Steroid, G – Glycoside S – Saponin, CA - Carboxylic acid and Q – Quinine. (– indicates absence of compounds)

Table.2 Antibacterial activity Standard control (Streptomycin)

S. No	Microbial strains	Standared control (Streptomycin) Inhibition zone (mm)
1.	*E. coli*	12
2.	*B. subtilis*	7
3.	*S. aureus*	9
4.	*P.aeruginosa*	11
5.	*K.pneumoniae*	10

Table.3 Antibacterial activity of methanol, ethanol, acetone, ethyl acetate, hexane, chloroform, petroleum ether, n-butanol, n-propyl alcohol, aqueous and diethyl ether extract of *T. chebula* Retz

S. No	Solvent	Microbial strains Inhibition zone (mm)														
		E. coli			*B. subtilis*			*S. aureus*			*P. aeruginosa*			*K. pneumoniae*		
		100 µl	150 µl	200 µl	100 µl	150 µl	200 µll	100 µl	150 µl	200 µl	100 µl	150 µl	200 µl	100 µl	150 µl	200 µl
1.	Methanol	11	12	13	9	10	11	9	12	14	10	11	12	11	13	17
2.	Acetone	12	14	16	10	12	14	13	14	15	13	14	15	11	13	16
3	Ethyl acetate	12	13	15	11	12	13	12	13	14	11	13	14	10	12	15
4.	Hexane	13	14	16	12	13	15	11	12	14	6	8	9	10	11	13
5.	Ethanol	11	12	17	11	13	15	10	12	15	12	14	16	11	13	14
6.	Petroleum ether	4	6	7	-	-	-	-	-	-	-	-	-	-	-	-
7.	Chloroform	-	-	-	-	-	-	-	-	-	3	4	5	-	-	5
8.	Diethyl ether	-	-	-	3	4	5	2	3	4	3	4	7	-	-	-
9.	n-propyl alcohol	9	11	12	9	10	12	9	11	12	8	10	13	7	9	11
10.	n-butanol	8	9	11	7	9	10	8	9	10	9	10	12	5	7	9
11.	Water	12	13	15	11	12	13	10	13	15	10	12	13	9	10	12

- indicates absence of zone formations

concentration (100µL, 150µL and 200µL) which produced inhibition zone of 4, 6 and 7mm respectively. (Table 2 and 3). Likewise, chloroform extract of *T. chebula* Retz. showed no activity against *E. coli*, *B. subtilis* and *S. aureus* but inhibited the growth of *P. aeruginosa* in all concentration and *K. pneumoniae* only with 200µL. It was found that diethyl ether extract of *T. chebula* Retz. gave inhibitory activity against *B. subtilis*, *S. aureus* and *P. aeruginosa* but not against *E. coli* and *K. pneumoniae*. n-butanol extract of *T. chebula* Retz. exhibited the antibacterial activity against all tested bacteria but the activity was less with *K. pneumoniae*. Water extract of *T. chebula* Retz. was more effective against *E. coli* than other bacteria. (Table 2 and 3)

Among the compounds isolated from *S. acuta*, its alkaloids appeared to be of great interest in pharmacological studies. These alkaloids belong to the family of indoloquinolines. Many investigations have been done on this family of compounds and the results showed that they are new leads in the establishment of drugs against many diseases. For example, cryptolepine 5-methylindolo-quinoline, the main alkaloid of the plant, has been well investigated for its various biological properties. First isolated from *Cryptolepis* species (*C. triangularis* and *C. sanguinolenta* (Periplocaceae) from Africa, the compound has been also isolated from other plants such as *S. acuta* (Malvaceae) from Sri Lanka (Gunatilaka *et al*., 1980), *Microphilis guyanensis*

(Sapotaceae) and *Genipa americana* (Rubiaceae) from Surinam (Yang *et al*., 1999). In Ghana, extracts of roots of *C. Sanguinolenta*, in which cryptolepine is the main alkaloid, have been used clinically to treat malaria, colic and stomach ulcers (Boye and Ampofo, 1983). Cryptolepine itself is found to produce many pharmacological effects such as antimicrobial (Cimanga *et al*., 1998), antiprotozoal, antihyperglycemic (Silver, 1993) and cytotoxic effects through GC-rich DNA sequence intercalation that provides basis for design of new anticancer drug (Guittat *et al*., 2003).

The present study is deals with the *T. chebula* Retz. was more effective in antibacterial activity of the eleven solvent used methanol, ethanol and acetone seems to be the best solvent when compare to other solvents. The extraction capacity of methanol, ethanol and acetone is higher than the other eight solvent used. This was understandable from the size of inhibition zone formed.

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