Phytochemical and Antimicrobial Evaluation of *Terminalia Chebula* Fruit Extracts

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Abstract-The present study includes the phytochemical and antimicrobial evaluation of the fruit of *Terminalia chebula* (harad) plant. Phytochemical screening of the fruit extract (shade dried) indicated the presence of flavonoids, terpenoids, tannins, alkaloids, saponin, carbohydrates, protein and glycosides. The antibacterial activity of methanol, ethanol, diethylether, acetone, chloroform and aqueous extracts of fruit of *Terminalia chebula* was evaluated against the human pathogenic bacteria like *Bacillus subtilis, Staphylococcus aureus, E coli, Pseudomonas fluorescens* and fungi like *Aspergillus niger, Aspergillus fumigates, Penicillium chrysogenum* by disc diffusion method. Among the extracts analyzed ethanol and acetone extracts showed promising results. The acetone fruit extract showed maximum inhibition against Gram positive bacterial strains *(Staphylococcus aureus,* 12.5mm; *Bacillus subtilis,* 12mm). Phytochemical tests carried out showed that the antimicrobial activity of plant *Terminalia chebula* fruit may be due to the presence of phytochemical compounds present in it.

Keywords: *Terminalia chebula,* Phytochemical , Antimicrobial

Introduction

Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances called phytochemicals include tannins, alkaloids, carbohydrates, terpenoids, steroids, flavonoids and phenols **(Edeoga *et al*, 2005).** Phytochemicals are chemical compounds produced by plants, generally to help them thrive or thwart competitors, predators, or pathogens **(Maria, 2017).** These phytochemicals have been used as poisons and others as traditional medicine **(Kannan, 2009).** *Terminalia chebula* (Harad) is one of the imperative herbs rich in medicinal properties used for digestive problems. Harad is a magical herb and its fruit is used to treat acidity, heat burn, heart disease, constipation, ulcers, piles, inflammation, dysentery and diarrhea. Harad is considered as a remarkable medicine for its healing properties. It is also used to remove toxins from body. It is good for lungs, bronchitis and sinus. Harad directly acts on the gastro-intestinal tract and reduces the passage of harmful toxins to the liver and kidneys **(*Tensingh* and *Astalakshmi,* 2014)**. It is one of the constituents of the popular Ayurvedic formula Triphala (which contains equal proportions of Harad (*Terminalia chebula*), Bahera (*Terminalia bellerica*) and Amla (*Emblica officinalis*). This herb is an effective

remedy for chronic ulcer, diarrhoea, dysentery and piles. It is also an effective purgative and helps in removing toxins and fats from the body. Harad contains chebulic acid, which catalyses the production of insulin generated by the pancreatic gland. It gives relief from the irritation and one is able to eat and chew with much less discomfort.(***Jigna Prakash*,2009)**

Material and Methods

The fruits of *T. Chebula* were collected from the local market in Muzaffarnagar, Uttar Pradesh. The taxonomic identity of the plant was confirmed by Dr. Sanjeev Kumar, plant taxonomist, Head of Botany Department, D.A.V. (PG), College, Muzaffarnagar, U.P., India.

Preparation of plant extract

Extracts were prepared according to the method described by **Ahmad and Beg, 1998** with minor modification. The samples were carefully washed under running tap water followed by sterile distilled water and air dried at room temperature (35-40°C) for 4-5 days, homogenized to a fine powder using a sterilized mixer grinder and stored in air tight bottles. Six different 50% solvents, namely ethanol, methanol, acetone, diethyl ether, chloroform and aqueous were used for extraction. Homogenized fruits, 10 g each (10%) were separately soaked in conical flasks each containing 100 ml of acetone, ethanol, methanol, acetone, diethyl ether, chloroform (50%) and sterile distilled water in conical flasks and allowed to stand for 30 min in a water bath (at 100°C) with occasional shaking, followed by keeping all the flasks on rotary shaker at 200 rpm for 24 h. 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 4°C in labelled steriled bottles until further use.

Phytochemical Screening

Extract of methanol, ethanol, acetone, chloroform, aqueous and diethyl were used for preliminary phytochemical screening using standard procedures. Phytochemical analysis for major phyto constituents of the plant extracts were 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, saponin, tannin, terpenoids, carbohydrates and proteins **(Harborne, 1973).**

Test for Tannins: 10 ml of bromine water was added to the 0.5gm aqueous extract. Decolouration of bromine water showed the presence of tannins.

Test for Saponins: 5.0 ml of distilled water was mixed with plants extract in a test tube and it was mixed vigorously. The frothing was mixed with few drops of olive oil and mixed vigorously and the foam appearance showed the presence of saponins.

Tests for Flavonoids: ***Shinoda Test***-Pieces of magnesium ribbon and HCl concentrated were mixed with aqueous crude plant extract after few minutes. The pink color showed the presence of flavonoid.

Alkaline Reagent Test- 2 ml of 2.0% NaOH mixture was mixed with aqueous plant crude extract; concentrated yellow colour was produced, which became colourless when we added 2 drops of diluted acid to mixture. This result showed the presence of flavonoids.

Tests for Glycosides: ***Liebermann's Test***- 2.0 ml of acetic acid and 2 ml of chloroform

were added with 10 ml plant crude extract. The mixture was then cooled and we added H_2SO_4 concentrated. Green colour showed the entity of a glycone, steroidal part of glycosides.

Keller-Kiliani Test: A solution of glacial acetic acid (4.0 ml) with 1 drop of 2.0% $FeCl_3$ mixture was mixed with the 10 ml plant extract and 1 ml H_2SO_4 concentrated. A brown ring formed between the layers which showed the entity of cardiac steroidal glycosides.

Salkowski's Test: 2 ml of concentrated H_2SO_4 was mix with plant crude extract. A reddish brown colour formed which indicated the presence of steroidal a glycone part of the glycoside.

Test for Terpenoids: 2.0 ml of chloroform was added with the 5 ml aqueous plant extract and evaporated on the water path and then boiled with 3 ml of H_2SO_4 concentrated. A grey colour formed which showed the entity of terpenoids.

Test for Alkaloids: Dragendroff's Test- Plant extracts were dissolved in chloroform. Chloroform was evaporated and the residue was acidified by adding few drops of Dragendroff's reagent (Potassium bismuth iodide). Appearance of orange red precipitate indicated presence of alkaloids.

Test for carbohydrates: Molisch's Test- 5 ml extract was mixed with 5 ml Molisch's reagent, and then 10 ml conc. H_2SO_4 was added along the sides of the test tube to form layers. Appearance of reddish violet ring the interference indicated the presence of carbohydrates.

Test for proteins: NinhydrinTest- Few drops of ninhydrin solution was added to the extract. Appearance of blue colour indicated presence of amino acid or proteins.

Antimicrobial Screening

Preparation of Culture Medium

Nutrient agar medium: 5gm of NaCl, 20gm agar, 10gm beef extract and 5gm peptone were added in 1000ml of distilled water which was boiled in a round bottom flask by continuous stirring. The mouth of the flask was wrapped with cotton plug and aluminium foil and tied tightly. The medium was sterilized at a pressure of 15 lbs and 121^{0}C for 15 minutes in an autoclaved and used for maintenance of bacterial culture and testing. **(Cappouccino and Sherman, 1998).**

Potato Dextrose Agar medium : 200gm of sliced peeled potatoes were boiled in 1 liter of water for 30 minutes. This mixture was filtered through cheesecloth, saving effluent, which is potato infusion. 20gm of dextrose, 20gm agar and water to effluent was added in it. The medium was sterilized at a pressure of 15 lbs and 121^{0}C for 15 minutes in an autoclaved and used for maintenance of fungal culture and testing.(**Cappouccino and Sherman, 1998).**

Microorganisms used: The bacterial strains and fungal strains were isolated from spoiled food and then identified the bacteria like *Bacillus subtilis, Staphylococcus aureus (Gram positive), E coli, Pseudomonas fluorescens (Gram negative)* and *fungi* like *Aspergillus niger, Aspergillus fumigates, Penicillium chrysogenum.*The bacterial strains were maintained in nutrient agar medium at 37^{0}C whereas fungal strain were maintained in potato dextrose agar medium at 28^{0}C. The stock culture slants were maintained at 4^{0}C.

Disc diffusion method

The antimicrobial test was carried out against gram positive and gram negative bacterial strains and some fungal strain. The antimicrobial activity of fruit extracts were tested against bacterial and fungal strains by disc diffusion method (**Cappouccino and Sherman, 1998; Mahato *et al,* 2005; Berghe and Vlietinck, 1991**). 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 and 28^oC for bacterial and fungal strains respectively for 24 hours. After the incubation, the diameter of zone (zone of inhibition) was measured. The results were recorded in millimeters (mm).

Results and Discussion

Phytochemical evaluation

In the present investigation it is indicated that the dried fruit of *T. Chebula plant* contained different types of phytochemicals such as alkaloids, flavonoids, saponin, terpenoids, tannin, glycoside carbohydrates and proteins in all the extracts. There have been reports that the presence of different phytochemicals with biological activity have valuable therapeutic index. It has been observed that the biologically active phytochemicals were present in all the extracts of *T. chebula*. (Table-1).The investigation showed that acetone and ethanol extract show the maximum qualitative results as compared to all other extracts. There has been report of the presence of different phytochemicals with biological activity that have valuable therapeutic index **(*Senthilkumar* and *Reetha*, 2009)**

Table-1 Phytochemical evaluation of different extracts of fruit of *Terminalia chebula*

S. No.	Phytochemicals	Extracts					
		aqueous	acetone	Methanol	ethanol	Di ethyl ether	chloroform
1	Saponin	+	-	-	-	-	-
2	Tannin	+	+ + +	+ +	+ + +	+ +	+ +
3	Flavonoid	+	+ + +	+	+ +	+	+
4	Terpenoid	+	+ + +	+ +	+ + +	+ +	+ +
5	Glycosides	+	+ +	+ +	+ + +	+ +	+
6	Alkaloid	+	+ + +	+ +	+ + +	+	+ +
7	Carbohydrates	+ +	+ + +	+ +	+ + +	+ + +	+ +
8	Protein	+ +	+	+	+	+	+

(+ = Presence of compound and - = Absence of compound)

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities (***Sofowora*,1993**). **Ali *SS et al*, 2008** reported that natural antioxidants mainly come from plants in the form of phenolic compounds, such as flavonoids, phenolic acids, tocopherols *etc.* The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper and inhibition of enzymes responsible for free radical generation **(*Benavente-garcia,* 1997**). 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 tannin. 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. Tannins are polyphenols that are obtained from various parts of different plants **(*Gajendiran and mahadevan*, 1990).**

Antimicrobial evaluation

In the present study, the antibacterial activity of the fruit of *Terminalia chebula* was tested by the disc diffusion method against bacterial species *like Bacillus subtilis, Staphylococcus aureus, E coli, Pseudomonas fluorescens and fungi like Aspergillus niger, Aspergillus fumigates, Penicillium chrysogenum.* The results of the present investigation indicated that the ethanol extracts of the dried fruit show maximum zone of inhibition against S*taphylococcus aureus* as well as *Bacillus cereus* (Gram-positive species) . *It also gives promising results against* Gram-negative bacteria which is used in this investigation (Table -2). The dried fruit showed higher zone of inhibition (12.5 mm in *Staphylococcus aureus* and 12 mm in *Bacillus sutilis)*. The aqueous extracts showed a decreased zone of inhibition when compared to all the extracts.

Table-2 Shows the antimicrobial evaluation (Inhibition zone in mm) of *Terminalia chebula* dried fruit in various extracts.

Strains		Extracts					
		Aqueous	Acetone	Methanol	Ethanol	Di ethyl ether	Chloroform
Gram+ve bacteria	*Bacillus subtilis*	5	12	10	11	10	9
	Staphylococcus aureus	4	12.5	8.5	10	7	8
Gram-ve bacteria	*E.coli*	3	11.5	8	12.5	9	11
	Pseudomonas fluorescens	2.5	11.5	11	12	9	10
Fungi	*Aspesgillus niger*	5,5	10	9	9	4	7.5
	Aspergillus fumigatus	4.5	9	9.5	8.5	7.5	8
	Penicillium chrysogenum	5	10.5	11	10	8.5	9.5

The presence of these phytochemical components may be responsible for the observed antimicrobial activity of the plant fruit extract. This finding conforms to the report of ***Anyanwu* and *Dawet*, 2005.** Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection**.** They have been found to be antimicrobial substances against wide array of microorganisms *in vitro*. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall **(*Marjorie*, 1999).** In addition to use in leather processing industries, tannins have shown potential of antiviral antibacterial (***Akiyama et al*, 2001),** and antiparasitic effects **(*Bhagavathi et al*, 1999)**. In the past few years tannins have also been studied for their effects against cancer through different mechanisms. Antibiotic resistance is a major concern and development of new agents from plants could be useful in meeting the demand for new antimicrobial agents with improved safety and efficacy **(*Thirupathy et al*, 2004).** Medicinal plants have been considered a boon to human society to cure a number of ailments **(Murray, 1995)**. Several works have documented the pharmacological screening of plant extracts which have been exploited as the source of innumerable therapeutic agents **(*Natrajan et al*, 2003; *Yoshikazu et al*, 2001; *Herrera et al*, 1996).**

Conclusion

Different types of secondary metabolites are reported from *Terminalia chebula* that have effective functions on many diseases. Especially the Terpenoids, Tannins, Flavonoids and Alkaloids are present in more quantity which makes this *plant* with high pharmacological properties. In the present study, it was found that *Terminalia chebula* acetone extract has an excellent antimicrobial activity. The pathogenic bacteria and fungi were inhibited in presence of the fruit extracts of *Terminalia chebula.* Therefore, the future studies should be aimed to exploit this plant to be used as one of the best medicinal plant for controlling pathogenic bacterial and fungal strains. Hence, it is considered as most important medicinal plant used in medicines of Ayurveda, Siddha, Unani and Homeopathy.

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