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INVITRO INVESTIGATION OF ANTIBACTERIAL, ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL SCREENING OF STEAM DISTILLED FRUIT EXTRACT OF *TERMINALIA CHEBULA*

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ABSTRACT

Nature has provided mankind with several plants which contain natural substances which cure diseases & promote health. Considering its medicinal property, extraction of oil from plant fruit materials of *Terminalia chebula* was used, as it proved to preserve the original qualities of the plant and also causes no degradation of the materials used. Steam Distillation process was used for the extraction of oil at lab scale using the available resources. The present study investigated, the phytochemical screening of *T chebula* extract, it revealed the presence of terpenoids, alkaloids,volatile acids and tannins .The antibacterial potential of fruit extract evaluated against clinical isolates showed the exhibition of antibacterial effect against all isolates. The antioxidant activity tested, showed a maximum inhibition in the range of 75-90% of 1mg/ml of extract and their IC_{50} value was found to be 620 µg/ml. The high content of total phenolic compound (440 µg GAE / mg of extract) revealed the antioxidant activity of the extract.

KEYWORDS: **Steam distillation, phytochemical screening, anti microbial activity, anti –oxidant activity, *Terminalia chebula***

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INTRODUCTION

Ayurveda is 5000 years old traditional knowledge of ancient Indian culture. Ayurvedic medicine is also referred to as "Mother of all healing" and is recently used by the western medical researchers[1].Though the use of ayurveda is invasive and less expensive compared to English medicines, its application is less due to the presence of some toxic chemicals. The purity obtained by the traditional methods makes it more susceptible for use because of some side-effects. Medicinal plants have been used to cure specific ailments from ancient times. This is passed over from generation to generation traditionally, all over the world. Nature has provided mankind with several plants which contains natural substances which cure diseases and promote health. In the past decades there is increased attention and interest in use of herbal medicines globally[2]. The World Health Organization reported that 80% of the world population relies chiefly on the usage of traditional medicines obtained from plant extracts or their active constituents[3]. Most of the medicinal plants have been used without any scientific evidence or background henceforth such treatments requires thorough scientific investigations to find out the pros and cons of the material being used [4]. Medicinal plants have become part of human society from the dawn of civilization to fight against diseases and are considered valuable and cheap source of many unique phyto constituents which are used extensively in the development and formulation of drugs against various diseases [5-7]. It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80%. Thus, the usage of medicinal plants is more in countries like India and few tribal regions than in other parts of the world. In the last few decades, the field of herbal medicine is getting popularized both in developed and developing countries [8]. This is because the herbal medicines are cheap and have natural origin with higher safety margins with lesser or no side effects [9]. *Terminalia chebula* is a moderate tree used in traditional medicines. It belongs to the family combretaceae[10] .It is found throughout India mainly in the region of deciduous forests and areas of light rainfall[11]. It is also known by the name Black myrobalan, Ink tree (or) Chebulic myrobalan [10].*Terminalia chebula* (Haritaki) has been extensively used in *Ayurveda, Unani* &Homeopathy medicine & has become cynosure of modern medicine [2]. Due to the wide spectrum of pharmacological activities associated with the biologically active chemicals present in this plant [10].The *Terminalia chebula* fruits possess diverse health benefits and have been used as traditional medicines as a house hold remedy [2]. It has a diverse range of medicinal properties like anti-oxidant, antibacterial antifungal,anti-neoplastic,antiviral,anti-diabetic,cardio-protective,Immunomodulatory,anti-analgesic,anti-carcinogenic,anti-helminthes,anti-mutagenic,anti-ulcer,anti-diabetic etc.[6] *T. Chebula is* also known for its anti-aging , immunity, body resistance against diseases and also used extensively in several Ayurvedic formulations prescribed for infectious diseases such as chronic ulcers, leucorrhoea, pyorrhoea and fungal infections of the skin[12]. It is Adapt genic and ant anaphylactic, Hypolipidemic, Hepatoprotective, Wound healing, Immunomodulatory and have Chemo preventive actions [13]. It is used for the treatment of a number of diseases like cancer, paralysis, cardio vascular diseases, ulcers, leprosy, arthritis, gout, epilepsy, Constipation, diarrhoea, ulcers, gastroenteritis, asthma, cough, dyspnoea, dyspepsia, haemorrhoids, candidasis, parasites, malabsorption syndrome, hepatomegaly, vesicular and renal stones, tumors, skin diseases, leprosy, intermittent fever, rheumatism , neuropathy, paralysis, memory loss, epilepsy, depression, diabetes, anorexia and wounds . As a home remedy it is advised by elders to keep a piece of *Terminalia chebula* fruit in the mouth as lozenge to suppress irritable cough which may be due its local anaesthetic action [14]. Recently, myrobalan (fruit of *T. chebula*), a component of

reputed ancient Indian herbal formulation "trifla" meaning three nuts (*T. chebula, Terminalia bellirica* (*Belliric myrobalan*) and *Emblica officinalis* dried nut) could effectively reduce genotoxicity of lead and aluminium in Allium test [15]. 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 [16]. The objective of this work is to determine the antimicrobial and antioxidant activity, phytochemical screening of steam distilled fruit extract of *Terminalia chebula*.

MATERIALS AND METHODS

The dried fruits of *Terminalia chebula* (myrobalan, alalekayi) was purchased from general stores, Bengaluru, washed with distilled water to remove excess dirt and then air dried. The fruit was powdered and sieved to screen the even size particles. 100g of this powder was taken in a flask to carryout steam distillation.

(i) Extraction of Plant

Steam distillation is a type of distillation or a technique used for separation of temperature sensitive materials like oils, resins, hydrocarbons, etc. which are insoluble in water or may decompose at their boiling point. Steam distillation enables a compound or mixture of compounds to be distilled at a temperature substantially below the boiling point of the individual constituents. Essential oils contain substances with boiling points up to 200°C or higher temperature in the presence of steam or boiling water. However, these substances are volatilized at a temperature close to 100°C and atmospheric pressure.

(ii) Experimental set up

The experimental set up is shown as in Fig 1. The experiment was conducted by constructing a prototype model that consist of two 1000ml and one 500ml conical flasks each of the conical flask mouth being closed by rubber cork with drilled holes for insertion of the connecting pipes of them connected together by connecting glass pipes. The first flask containing water was continuously boiled for generation of steam, the second one containing the sample and the third one for collecting the condensate, each of them being connected in series.

Figure 1
the experimental set up for steam distillation on lab scale.

(iii) Phytochemical Screening

The phytochemical screening of extract was done to identify the main groups of Chemical constituents present in extracts of *T. chebula* by their color reaction [6, 7]. Saponin test: 2.5 ml of the sample was added to 10ml of distilled water and shaken vigorously for 30 seconds. The sample was allowed to stand for 1 hour and observed for froth formation. Terpenoids test: 0.5 ml of sample was taken and 2ml of chloroform was added to it. Conc.H_2SO_4 (3 ml) was slowly added from the sides of the test tubes and observed for formation of layer at interface. Alkaloids test: 15 ml of sample was added with 2ml of ammonium hydroxide and extracted 3 times with 10ml of chloroform. The chloroform layer was separated and washed 3 times with 2 ml of HCl. The sample was now separated into two parts and tested with Mayer's reagent and Wagner's reagent for the presence of Alkaloids. Volatile oils: 2ml of sample was added with 0.1 ml of 0.1N NaOH and a small quantity of dil.HCl was added and observed for formation of white precipitate. Tannins: The extract was diluted with water and 3-4 drops of 10% $FeCl_3$ was added and observed for formation of either blue or green color to detect the presence of tannins.

(iv) Anti- bacterial activity by disc diffusion method

Whatmann filter paper was used to prepare discs and sterilized before using. Micro-organisms chosen: *E.Coli, Shigella, Salmonella, S.aureus, Vibrio cholera* Nutrient broth was prepared and autoclaved. A loopful of bacterial colony was taken and inoculated in the nutrient broth and incubated for 48 hours. Nutrient agars was prepared and poured in the sterilized petriplates and bacterial culture was spread on the plate. The discs which were loaded with the plant extract samples and allowed to stand for 2 hours were placed at their respective positions and incubated at 37 deg Celsius for 3-4 days[20, 21]

(v) Antioxidant Assays

Sample are dissolved in 95% methanol to make a concentration 1mg/ml and diluted to prepare series of concentration for antioxidant assay.

Determination of total phenol content

The total phenolic content of T.chebula was estimated by spectrophotometric method. 1 ml of sample (Concentration 1 mg/ml) was mixed with 1 ml of Folin-Ciocalteu's phenol reagent. After 3 minutes, 1 ml of2% sodium carbonate solution was added to the mixture and made upto 10 ml with distilled water. The reaction was kept in the dark for 90 minutes, after which the absorbance was read at 725 nm. Gallic acid was used for constructing the standard curve (200-1000 µg/ml) and the results were expressed as µg of gallic acid equivalents/mg of extract (GAEs) [22].

Hydrogen peroxide scavenging test

An aliquot of H_2O_2 (2mM) and various concentrations (100-1000 µg/ml) of samples were mixed (1:0.6 v/v) and incubated for 10 min at room temperature. After incubation, absorbance of H_2O_2 at 230 nm was determined against a blank solution containing H_2O_2. For each concentration, a separate blank was used for background subtraction [23].

Scavenging activity of H_2O_2

% scavenging activity [H_2O_2] = Abs [control] – Abs [Sample]/ Abs [control] X100

Abs [control]: absorbance of H_2O_2 as control

Abs [sample]: absorbance of Sample (extract)

Reducing power

Different concentration of sample(0.2 ml and 0.4ml) in 1ml of DDW was mixed with phosphate buffer (2.5ml,0.2M,pH 6.6) and [$K_3Fe(CN)_6$] (2.5ml,1%). Incubate for 20 mins at 50 deg Celsius. A portion (2.5ml) of TCA (10%) was added to the mixture. Centrifuge at 3000rpm for 10 min. the upper layer (2.5ml) was mixed with DDW(2.5ml) and $FeCl_3$(0.5ml,0.1%). Absorbance was measured at 700nm. The increased absorbance is an indication of increased reducing power. Ascorbic acid was used as reference standard and phosphate buffer (pH 6.6) was used as blank solution [23].

RESULTS

1. Preliminary phytochemical screening

Table 1
Phytochemical analysis of Extracts of Terminalia chebula

Sl.No.	Phytochemical constituents	Extract of TC
1	Saponins	-
2	Terpenoids	+
3	Alkaloids	+
4	Volatile acid	+
5	Tannins	+

Phytochemical screening of the extract reveals that the presence of terpenoids, tannins alkaloids, phenolic compounds are most prominent

2. Antibacterial activity of extract

Table 2
Antibacterial activity of extract of T. Chebula

Sl.No.	Organism Tested	Diameter of inhibition zone in mm
1	*S.aureus*	+(11mm)
2	*E.coli*	+(10mm)
3	*S.typhi*	+(10mm)
4	*Shigella*	+(14mm)
5	*V.Cholerae*	+(15mm)

Extracts of T .chebula shows the different level of inhibition against the bacterial isolated such as S.aureus, Shigella, V.Cholera, E.coli salmonella

3. Anti oxidant studies
a) Determination of total phenolic content

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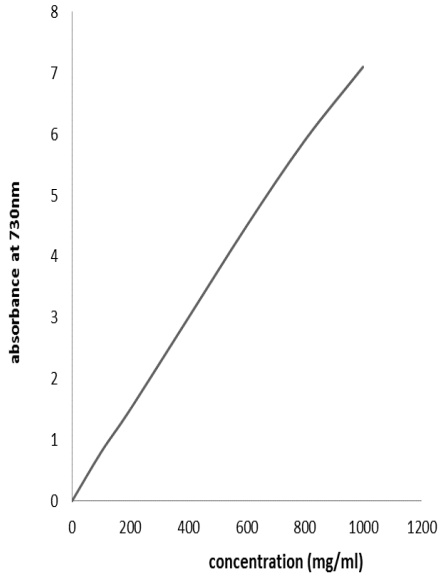
Graph 1
Total phenolic content of Terminalia chebula extract

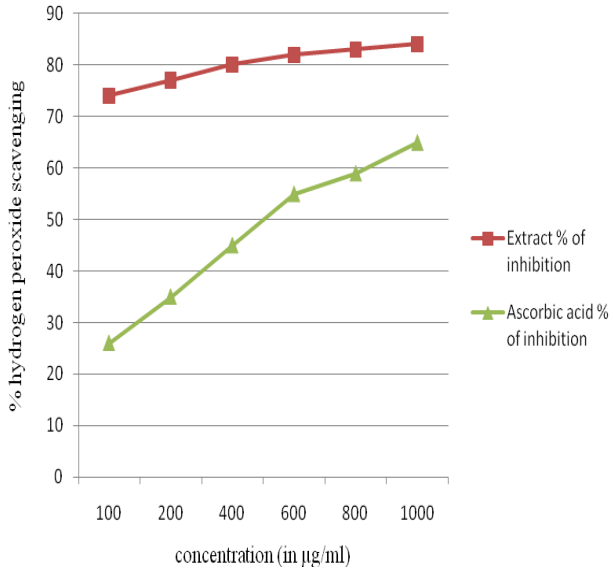


The value obtained for the T.chebula extract is plotted on standard GAE to determine the total phenolic content in the extract.

Phenolic compounds are contributing to the antioxidative action. The total phenol content was found to be 440 µg equivalents to gallic acid content of gram of extract at absorbance of 725nm.The antioxidant effect of extract of TC is due to presence of phenol compound .In this respect polyphenolic compound such as phenolic acid commonly found in plant have been reported to have biological effect includes antioxidant effect [23].

b) Hydrogen peroxide scavenging activity

Graph 2
H_2O_2 scavenging activity

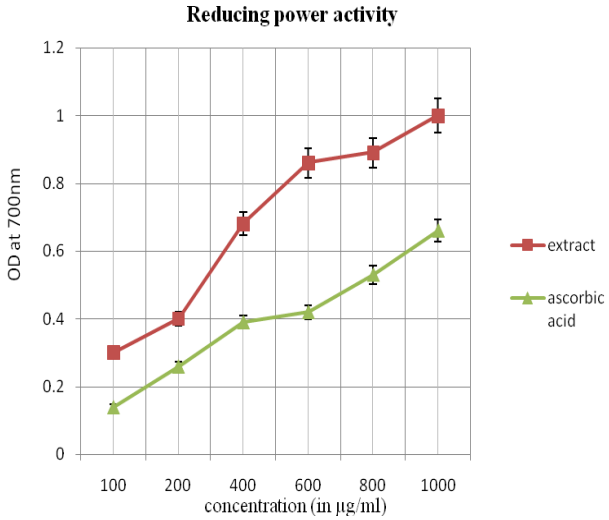


Graph represents, steam distillated extracts is a good scavenger of H_20_2 (IC_{50}= 620 µg/ml) Compared with standard ascorbic acid (IC_{50}= 490µg/ml)

The dose inhibition curve and IC_{50} value of fruit are shown in the graph. The maximum inhibition was in the range of 75-90% in presence of 1mg/ml of extract. The explanation for higher IC_{50} value (IC_{50}= 490 µg/ml) was found in this experiment. Result shows that it has high scavenging activity has previously reported [23].

c) Reducing power assay

Graph 3



Graph represents reducing power activities of the extract of T.chebula in comparison with a standard ascorbic acid

The reducing capability of extract were compared with standard ascorbic acid as shown in graph 3.The reducing capability of extract gradually increases with a rise in concentration. As shown in the figure 2, Fe^{3+} was transformed to Fe^{2+} in the presence of extract and the reference compound ascorbic acid to measure the reductive capability. At 0.2mg/ml the absorbance was 0.32 and 0.018 respectively at 700nm, while at 1mg/ml the absorbance of extract was more than Standard ascorbic acid, it showed that extract have maximum reducing capability when compared with the standard.

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DISCUSSION

Our result showed that extract of *T chebula* have phytochemical properties. Phytochemical screening reveals the chemical constituent of the plant extract(table1) and also plant extract shows highest antibacterial activity for *S.aureus, Shigella, V.Cholera, E.coli* and *Salmonella* (table2).In antioxidant studies that the extract should posses the ability of inhibiting free radical formation or itself be a free radical scavenger. The dose inhibition curve and IC_{50} value of fruit are shown in fig the maximum inhibition was in the range of 75-90% in presence of 1mg/ml of extract. The explanation for higher IC_{50}value (IC_{50}=620µg/ml) was found in this experiment(graph2) .The antioxidant effect of extract of *T chebula* is due to presence of phenolic compound .In this respect polyphenolic compound such as phenolic acid commonly found in plant have been reported to have biological effect includes antioxidant effect. It was reported that extract has high phenolic content of 440µg equivalent to gallic acid content of gram of extract(graph1).In this experiment extract also measured for their reductive ability it has been found that there is transformation of fe3+ - fe2+ in the presence of plant extract. And the presences of reductones are associated with reducing properties (graph3).

CONCLUSION

The *Terminalia chebula* extract shows a phytochemical properties, antibacterial activity, antioxidant activity by inhibiting hydrogen peroxide and the reducing power activity was detected when compared with standard ascorbic acid .In addition *T.chebula* fruit extract also contains phenol compounds that are measured equivalent to gallic acid content of gram of extract.

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