ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 9, Issue 6, 2016

Online - 2455-3891
Print - 0974-2441

Research Article

PHYTOCHEMICAL ANALYSES AND EVALUATION OF ANTIOXIDANT, ANTIBACTERIAL AND TOXIC PROPERTIES OF *EMBLICA OFFICINALIS* AND *TERMINALIA BELLIRICA* FRUIT EXTRACTS

HIMANI BADONI, PROMILA SHARMA, SYED MOHSIN WAHEED*, SAUMYA SINGH

Department of Biotechnology, Graphic Era University, Clement, Dehradun, Uttarakhand, India. Email: syedmohsinwaheed@yahoo.com

Received: 27 June 2016, Revised and Accepted: 04 July 2016

ABSTRACT

Objective: The present study deals with the investigation and evaluation of phytoconstiuents of *Emblica officinalis* and *Terminalia bellirica* fruit extracts for their antibacterial and antioxidant potential, as well as their potential acute toxicity.

Methods: Phytochemical analyses was performed besides antioxidant activity of the extracts was alaso determined using diphenylpicrylhydrazyl (DPPH), total phenolic content (TPC), total flavonoid content (TFC) assays. Antimicrobial activity of the extracts was evaluated by agar well diffusion assay.

Results: The extracts prevented the growth of both Gram-positive and Gram-negative bacteria. The *E. officinalis* aqueous fruit extract (EOA) and *E. officinalis* methanolic fruit extract (EOM) both exhibit tannins, saponins, phenols, and carbohydrates, whereas alkaloids and flavonoids are found only in EOM, and glycosides showed their presence in aqueous extract only. In contrast, *T. bellirica* aqueous fruit extract (TBA) and *T. bellirica* aqueous fruit extract (TBM) both contains alkaloids, tannins, coumarins, flavonoids, and phenols. While carbohydrates are present barely in TBM, TBA extract showed the presence of saponins and glycosides. The TPC results show that EOM (528±0.013) has the highest phenolic content in it followed by EOA (509±0.003), TBM (284±0.06), and TBA (280±0.036). In TFC analysis too, EOM (154±0) showed a higher concentration range of flavonoids followed by EOA (142±0), TBM (126±0.017), and TBA (119±0.007). In DPPH scavenging assay, EOM (86.88±0.310) showed maximum % inhibition followed by EOA (85.32±0.414), TBM (80.57±0.569), and TBA (75.55±0.362), respectively. Antibacterial assay showed varying results for different bacterial strains such as, for EOA (10±1) demonstrated highest inhibition against *Escherichia coli*, EOM (11.5±0.5) exhibited maximum inhibition against *Staphylococcus aureus*, for *Klebsiella pneumoniae* EOA (13±2) displayed maximum inhibition and in case *Pseudomonas aeruginosa* too EOA (12.5±0.5) showed maximum inhibition while TBM and TBA too showed significant amount of inhibition against test bacterial strains. The antibacterial and antioxidant activities of the extracts were found to be positively associated with the TPC and TFC of the extracts. This study was conducted to identify the phytochemical composition of the fruit extracts of the two plants, their antioxidant and antibacterial potential, along with their neuroprotective and nootropic role. To establish their neuroprotective role, acute toxicity study was conducted on Wistar rats to establish the safety of the extracts.

Conclusion: The current study demonstrates and compares the antioxidant and antibacterial activities of the fruit extracts of *T. bellirica* and *E. officinalis* and acute toxicity study further demonstrates that the extracts used are safe to conduct neuroprotective studies.

Keywords: Total phenolic content, Total flavonoids content, *Emblica officinalis* methanolic fruit extract, *Emblica officinalis* aqueous fruit extract, *Terminalia bellirica* methanolic fruit extract, *Terminalia bellirica* aqueous fruit extract.

 DOI: http://dx.doi.org/10.22159/ajpcr.2016.v9i6.13731

INTRODUCTION

Since long times, herbal medications have been used for the treatment of various disorders. As traditional herbal medicines have been in wide use since ancient times, many treatments are known to be effective against different types of diseases. Despite the fact, toxicity of plant-derived medicines has not been determined as it is generally assumed that medicines derived from plants are safer than synthetic drugs. Most of the plants possess antioxidant activity or natural antioxidants which are helpful in reducing oxidative stress [22]. Oxidative environment presents in the living organism comprise superoxide, hydroxyl radical, nitric oxide, and peroxynitrite. These free radicals are the cause of various diseases namely cancer, neurodegeneration, and diseases resulting from inflammation. Antioxidants have the ability to counter the effects of free radicals. Various medicinal plants throughout the world are being explored for their antioxidant, anticancer, and antimicrobial properties and therapeutic potential [32]. The current study deals with the analysis of total phenolic content (TPC) and total flavonoid contents (TFCs), along with an evaluation of antioxidant and antibacterial activities of methanolic and aqueous extracts of *Emblica officinalis* and *Terminalia bellirica* fruits.

E. officinalis, widely known as amla, used as a "rejuvenating herb" in Ayurveda since ancient times. *E. officinalis* extracts contain various antioxidants such as emblicanin A, emblicanin B, gallic acid, ascorbic acid, ellagic acid which are effective in a range of ailments. Presence of various active phytocompounds bestows Emblica officinalis fruit eaxtract with various therapeutic benefits consequential of radioprotective [14], antiatherosclerotic [20], antidiabetic [21,34], antiaging [38], gastroprotective [1], cytoprotective and immunomodulatory [29] properties (Fig. 1).

T. bellirica is known as Vibhitaki in Sanskrit, meaning impudent. It contains a vast range of important phytoconstituents such as bellericanin, ellagic acid, gallotannic acid, gallic acid, termilignan, tannins, ellargic acid, ethyl gallate, galloyl glucose and chebulaginic acid, phenyllemblin, mannitol, glucose, fructose, and rhamnose. These phytocompounds were found to be responsible for numerous pharmacological activities such as antimicrobial, antioxidant, antidiarrheal, antidiabetic, antihypertensive, antisalmonella, antispasmodic, antimalarial, antibacterial, anti-HIV, antifungal, antimutagenic beside being analgesic, hepatoprotective, and bronchodilatory in action [2]. In addition to this, the plant is used in the treatment of gastric ulcer, constipation, general weakness, and

Fig. 1: *Emblica officinalis* (amla)

Fig. 2: *Terminalia bellirica* (baheda)

piles. Hence, this plant plays a significant role in the prevention and treatment of various tyes of diseases [15]. Antiproliferative effects on several cancer cell lines have also been reported [16] (Fig. 2).

Bacteria cause numerous diseases as a result of pathogenic activity or opportunistic pathogenesis. Complications from bacterial infections may result in the breakdown of blood brain barrier (BBB) and result in various neurological pathologies or neuropathies.

Currently, there is a surge in the multi-drug resistance strains of pathogenic bacteria. Thus, there is an urgent need to explore safe and alternate medicines/medications from natural sources such as plants. Medicines from plants can be used alone or along with conventional drug regimens to enhance their efficacy. Quite often anti-inflammatory medicines that are used to treat infections cause breakdown of BBB. Thus, medicines from plants used in Indian traditional medicine known to have proven efficacy and safety could be suitable for this purpose. *T. bellirica* and *E. officinalis* are the two plants known to have immense medicinal value and benefits. *Terminalia chebula* has already been much explored for its medicinal value, while *T. bellirica* that belongs to the same genus is less studied and thus less explored for its medicinal value. Current study deals with the analysis of phytochemical composition, evaluation of their antioxidant and antibacterial potential, along with the neuroprotective role of the fruit extracts of two plants. Consequential to this, study also demonstrates the safety and suitability of these extracts to carry out further studies and establish their neuroprotective role, a preliminary acute toxicity study was conducted on Wistar rats.

Central nervous system (CNS) infection starts with invasion through a adjacent structures, or directly as a result of head trauma, surgery or invasive diagnostic procedures. [17]. Numerous bacteria grow in the body of healthy people which do not harm usually, but some rare strains can cause serious disease. The vast majority of cases are caused by disease-causing strain. Adults and older children never get affected by *Escherichia coli* meningitis, until they have health problems which destroy their immune system, or any injury to the head results in entry of bacteria through the wound [36, 40].

Reports indicate that hematogenous spread of bacteria from a primary site of infection has the ability to cross BBB to pierce the CNS and persist in the brain despite clearance from the bloodstream [35]. Infections in patients with impaired host defense mechanism have increasing susceptibility to brain abcess. Gas forming organisms are the ones that generally lead to brain abscess [18].

The microorganisms used in this study, viz., *E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Staphylococcus aureus* are responsible for various infections and complications resulting from these infections leading to the breakdown of BBB. Thus, compromising the privileged immune status of CNS.

MATERIALS AND METHODS

Collection of plant material

The fresh fruits of *E. officinalis* and *T. bellirica* were collected from the Dehradun region of Uttarakhand. The taxonomic identification of plant was confirmed by Dr. S. K. Srivastava, Scientist "D" from Botanical Survey of India, Dehradun, Uttarakhand. The accession number of *E. officinalis* (*Phyllanthus emblica* L.) is 114632 and *T. bellirica* (Gaertn.) Roxb is 115221.

Preparation of extracts

Fruits of *E. officinalis* (amla) and *T. bellirica* (bahera) were washed under running tap water and chopped into fine pieces; seeds were removed and fruits were air dried at room temperature (RT). The dried fruits were grind to fine powder using a grinder and stored in air tight bottle. 10 g of *E. officinalis* powder was separately soaked in two sterile containers containing 100 ml of methanol and 100 ml sterile distilled water. Both containers were subjected to cold maceration (occasional shaking and it was kept undisturbed for 48 hrs). Both were centrifuged at 3000 rpm and supernatant was dried and stored at 4°C [10,4,33].

Preliminary phytochemical analysis

Crude plant extract samples were dissolved in suitable solvents and used for qualitative analysis of key phytochemical constituents such as alkaloids, flavonoids, phenolics, saponins, steroids, tannins, proteins, quinones coumarins, glycosides, and carbohydrates. Qualitative phytochemical analyses of *E. officinalis* and *T. bellirica* were performed according to the methods of [30,31,25].

Test for detection of alkaloid

1 ml of extract was taken in a test tube, followed by addition of 1 ml of Wagner's reagent. Appearance of brown flocculent and precipitation reveals the presence of alkaloids.

Test for detection of tannins

1 ml of the extract was taken in a test tube, and then 1 ml of 0.1% ferric chloride containing 0.1 N HCl was added. Appearance of blue-black coloration indicates the presence of tannins.

Test for detection of proteins

Few drops of ninhydrin reagent were added to the 1 ml of extract. The appearance of purple color indicates the presence of proteins.

Test for detection of saponins

Extract was mixed with 5 ml distilled water and then agitated in a graduated cylinder for 15 minutes. Formation of foam indicates the presence of saponins.

Test for detection of quinones

To 1 ml of plant extract, 1 ml concentrated H_2SO_4 was added. Blue-green or red coloration indicates the presence of quinones.

Test for coumarins

About 1 ml of 10% NaOH was added to the 1 ml extract. Appearance of yellow shows the presence of coumarin.

Test for phenols
Few drops of ferric chloride were added to 1 ml of extract in a test tube. Development of dark green indicates the presence of phenols.

Test for flavonoids
To 1 ml of extract, 1 ml of dilute ammonia solution was added followed by the addition of concentrated H_2SO_4 dropwise. A yellow coloration observed indicates the presence of flavonoids.

Test for carbohydrates
Two test tubes were taken, one containing 1 ml of the extract to be tested and second tube containing 1 ml of glucose was used as a control. 1 ml of Fehling's Reagent was added to both the samples. Appearance of green, yellow, or red in plant extract reveals the presence of reducing sugars.

Test for glycosides (Keller-Kiliani test)
About 1 ml of extract was treated with 1 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlaid with 1 ml of concentrated H_2SO_4. A brown ring at the interface indicates the presence of glycosides.

Test for sterols
About 2 ml of acetic anhydride was added to 1 ml crude extract of plant sample with 2 ml H_2SO_4. The color change from violet to blue or green indicates the presence of sterols.

Determination of TPC
To determine the TPC of *E. officinalis* and *T. bellirica* extracts, Folin-Ciocalteu method was used [13]. To 150 µl sample standard or a positive control, 500 µl of 1:10 Folin –Ciocalteau phenol reagent was added. The mixture was allowed to stand for 5 minutes before the addition of 350 µl of 10% sodium carbonate (Na_2CO_3). The resulting reaction mixture was incubated in the dark at RT for a further 2 hr period. Absorbance was then measured at 765 nm using a spectrophotometer. 1 ml of standard solutions of concentrations 0.2, 0.4, 0.6, 0.8, and 1 mg/ml of gallic acid were prepared in methanol. Concentration of 2 mg/ml of plant extracts was also prepared in methanol. Results were expressed in milligrams of gallic acid equivalents (GAE) per gram of dried extract.

Determination of TFC
For flavonoid content determination, aluminium chloride method was used. Total flavonoid contents were measured as quercetin equivalent (QE) per gram of dried extract by comparing with quercetin. Standard curve of quercetin was drawn to compare and calculate QE of the extracts. Standard or extract solution (0.2-1 mg/ml) was taken into 1 ml volume, containing 160 µl of distilled water, 15 µl of 5% $NaNO_2$, and 100 µl plant extract. After 5 minutes, 15 µl 10% $AlCl_3$ was added to the mixture. At the 6th minutes, 100 µl of 1M NaOH was added, and volume made up to 1 ml with distilled water. The absorbance was measured using UV-visible spectrophotometer at 510 nm.

1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity
To evaluate the antioxidant property of extracts, DPPH radical scavenging method developed by Blois was used. For this purpose, the antioxidant activity of the extracts was compared with the natural antioxidant, ascorbic acid. Stock solution of DPPH assay was prepared by dissolving 24 mg DPPH with 100 ml methanol and then stored at −20°C for further use. The working solution was prepared by adding 10 ml of the stock solution to 45 ml methanol to obtain an absorbance of 1.10±0.02 units at 515 nm. Add 150 µl (1 mg/ml) of fruit extract solution to react with 2850 µl of the DPPH solution for 30 minutes in the dark and absorbance was measured at 515 nm [7,37,3].

The solution without any extract with DPPH and methanol was used as control. Ascorbic acid was used as standard. Inhibition of DPPH free radical in percentage was calculated by the formula:

$$\text{Inhibition (\%)} = [(A_{control} - A_{test})/A_{control}] \times 100$$

Where, $Abs_{control}$ is the absorbance of DPPH radical in methanol; Abs_{sample} is the absorbance of DPPH radical solution mixed with sample extract/standard [12].

Antibacterial assay
Preliminary antibacterial activity of methanolic and aqueous extracts of *E. officinalis* and *T. bellirica* fruits was done by agar well diffusion method [26]. The bacterial strains were taken for studies are *E. coli* (MTCC 1698), *S. aureus* (MTCC 6908), *K. pneumoniae* (MTCC 9544), and *P. aeruginosa* (MTCC 4306). On Luria Bertani agar 100 µl of the appropriate bacterial suspension was inoculated using sterile swabs. 20 µl of each extract was added to the 5 mm wells, and the plates were incubated to allow the diffusion of the extract. DMSO was taken as negative control and ampicillin was taken as standard antibiotic. Clear area devoid of microbial growth was measured to determine antibacterial activity. The diameter of the zone of inhibition was taken as mean of three replicatesmean of two replicates±standard error of the mean (SEM).

Preliminary toxicity study

Experimental animals

Male Wistar rats, 7-week-old and weighing 100-200 g, were used for the present study. They were purchased and maintained in the animal house of Pinnacle Biomedical Research Institute, Bhopal, Madhya Pradesh, India, for experimental purpose. The experimental protocol was approved by the Institutional Animal's Ethics Committee and by the regulatory body of the government (Reg. No. 1824/PO/ERe/5115/CPCSEA and Protocol approval number is PBRI/IAEC/PN-017). The animals were maintained in suitable conditions of temperature (24°C±2°C), humidity (45%±5%), and 12 hr light-dark cycles. It is necessary that all the animals were acclimatized for 7 days before the study. The animals were grouped into experimental and control groups. They were housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water *ad libitum*. Animals were habituated to laboratory conditions for 48 hr before experimental protocol to minimize if any of non-specific stress. All experimental protocols were followed as per the Ethics Committee on Research in Animals.

Acute toxicity study
The acute toxicity of *E. officinalis* and *T. bellirica* methanolic extracts was determined. Rats were fasted overnight and randomly divided into six rats per group. Extract doses were given as per OECD guidelines (5, 50, 300, 1000, and 2000 mg/kg body wt.) and were administered separately to the rats of each group using bulbed steel needle. All rats were then allowed free access to food and water and observed over a period of 48 hr for signs of acute toxicity. The number of deaths within this period was recorded [23].

Statistical analysis
All the experiments were repeated thrice to obtain independent observations. Data from laboratory studies were analyzed separately for each experiment and were subjected to one-way analysis of variance using SigmaStat®3.5 Software followed by Bonferroni *t*-test ($p \leq 0.001$). Data are expressed as mean±SEM.

RESULTS

Phytochemical analysis
Preliminary phytochemical analyses determine that both the methanolic and aqueous extracts of *E. officinalis* possess tannins, saponins, phenols, and carbohydrates. Alkaloids and flavonoids are present only in methanolic extract, whereas glycosides are present only in aqueous extract. Although, in *T. bellirica,* alkaloids, tannins, coumarins, flavonoids, phenols are present in both methanolic and aqueous extracts. Carbohydrates are present only in methanolic extract, whereas aqueous extract showed the presence of saponins and glycosides. The results of phytochemical analyses are shown in Table 1.

TPC

TPC demonstrated the presence of phenolic compounds present in various concentrations in different extracts of *E. officinalis* and *T. bellirica*. Methanolic extract of *E. officinalis* fruit extract showed the highest concentration of phenolics (528±.013 mg/GAE g dry wt) among all the four extracts, followed by aqueous extract (509±0.003 mg/GAE g dry wt) of the same. However, in *T. bellirica*, methanolic fruit extract showed higher concentration/amount (284±0.06 mg/GAE g dry wt) than aqueous extract (280±0.036 mg/GAE g dry wt) as shown in Fig. 3.

Table 1: Preliminary identification of phytochemical constituents from *Emblica offcinalis* and *Terminalia bellirica* fruit extracts

Phytochemicals	Methanolic (EOM)	Aqueous (EOM)	Methanolic (TBA)	Aqueous (TBM)
Alkaloids	+	-	+	+
Tannins	+	+	+	+
Proteins	-	-	-	-
Saponins	+	+	-	+
Quinones	-	-	+	-
Coumarins	-	-	+	+
Flavonoids	+	-	+	+
Phenols	+	+	+	+
Carbohydrates	+	+	+	-
Glycosides	-	+	-	+
Sterols	-	-	-	-

+: Present, -: Absent, EOA: *Emblica offcinalis* aqueous fruit extract, EOM: *Emblica offcinalis* methanolic fruit extract, TBA: *Terminalia bellirica* aqueous fruit extract; TBM: *Terminalia bellirica* methanolic fruit extract

TFC

The TFC in different extracts presents in various concentrations. Flavonoid content is highest in methanolic extract of *E. officinalis* fruits (154±0 mg/QE g dry wt) among all the extracts, followed by aqueous extract (142±0 mg/QE g dry wt) of same, then methanolic fruit extract of *T. bellirica* (126±0.017 mg/QE g dry wt) showed higher concentration than aqueous (119±0.007 mg/QE g dry wt) fruit extract as shown in Fig. 4.

Antioxidant (DPPH) assay

DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The decline capability of DPPH radical is determined by the decrease in absorbance at 515 nm induced by antioxidants. The extracts are able to reduce the stable radical DPPH to the yellow-colored diphenyl picrylhydrazine. In this context, the highest free radical scavenging activity was shown by EOM (86.36%) followed by EOA (85.32%), TBM (80.15%), and TBA (76.02%) as shown in Fig. 5.

Antibacterial assay

Growth inhibition was exhibited by aqueous and methanolic fruit extracts of *E. officinalis* and *Terminalia bellerica* toward bacterial strains. These results determine that EOA showed maximum zone

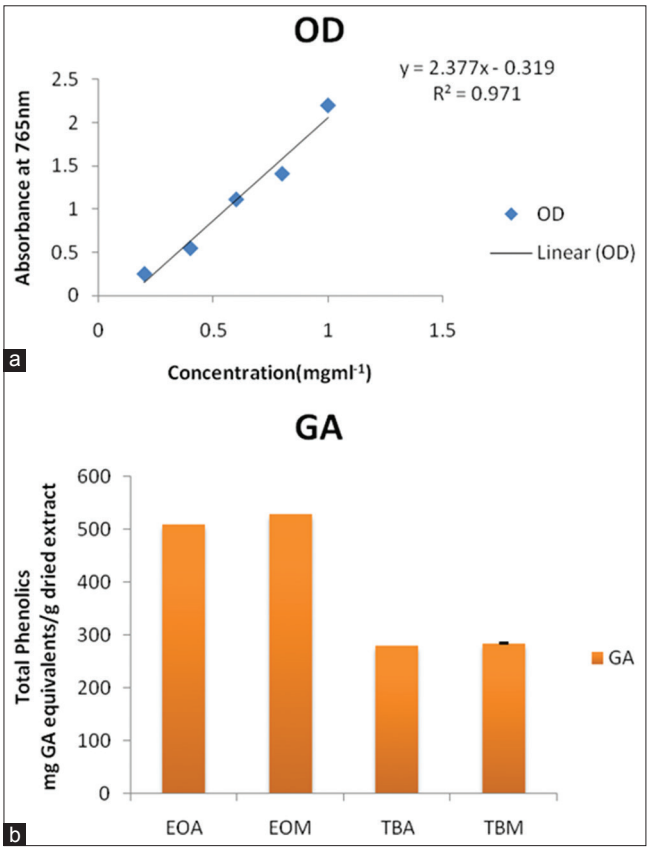

Fig. 3: (a and b): Quantification of phenols. Total phenolic content of methanolic and aqueous extracts of *Emblica officinalis* and *Terminalia bellirica*. Each value is expressed as the mean±standard error. There is a statistically significant difference (p≤0.001). EOA: *Emblica offcinalis* aqueous fruit extract, EOM: *Emblica offcinalis* methanolic fruit extract, TBA: *Terminalia bellirica* aqueous fruit extract, TBM: *Terminalia bellirica* methanolic fruit extract

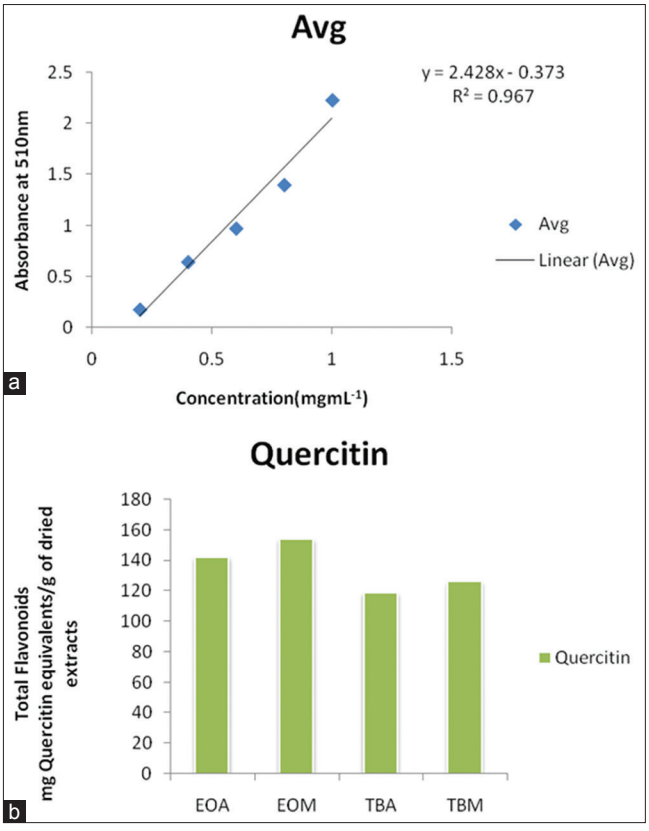

Fig. 4: (a and b) Quantification of flavonoids. Total flavonoids of methanolic and aqueous extracts of *Emblica offcinalis* and *Terminalia bellirica*. Each value is expressed as the mean±standard error. There is a statistically significant difference (p≤0.001). EOA: *Emblica offcinalis* aqueous fruit extract, EOM: *Emblica offcinalis* methanolic fruit extract, TBA: *Terminalia bellirica* aqueous fruit extract, TBM: *Terminalia bellirica* methanolic fruit extract

of inhibition against *E. coli* (10±1 mm) followed by EOM and TBM (9±1 mm) have the same range, with minimum inhibition for TBA (8.5±0.5 mm). For *S. aureus*, the maximum zone of inhibition was shown by EOM (11.5±0.5 mm) followed by EOA and TBA (10.5±0.5 mm) with same inhibition range, although TBM (9.5±0.5 mm) showed minimum inhibition. For *K. pneumoniae,* maximum zone of inhibition against showed by EOA (13±2 mm), followed by EOM (12.5±0.5 mm), TBM (12±1 mm), and then TBA (11.5±0.5 mm). In *P. aeruginosa*, maximum zone of inhibition was shown by EOA (12.5±0.5 mm) followed by EOM (12±2 mm), TBA (11.5±0.5 mm), and then TBM (10.5±0.5 mm). Although the extracts of *E. officinalis* and *Terminalia bellerica* posses significant zone of inhibition as compared to standard antibiotic (ampicillin). The results are summarized in Figs. 6 and 7.

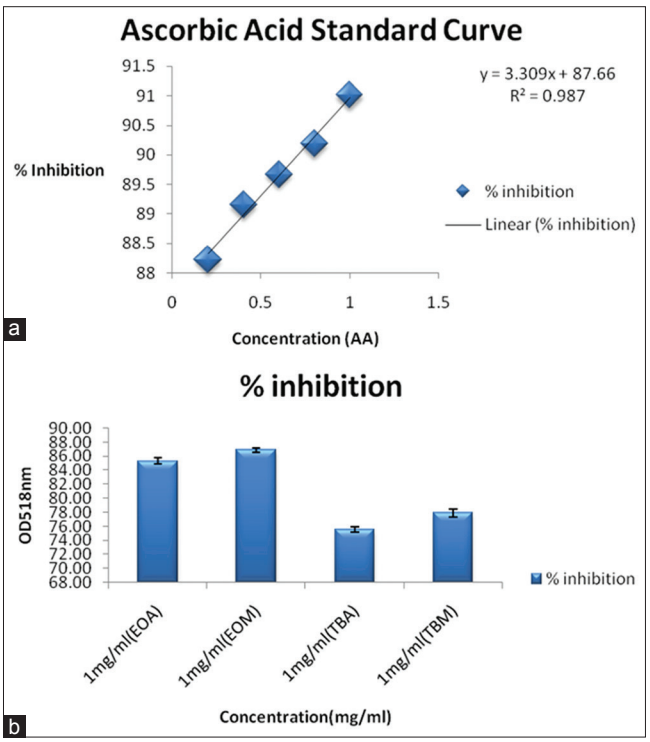

Fig. 5: (a and b) 1,1-diphenyl-2-picrylhydrazyl (DPPH) % inhibition. Free radical scavenging activity of methanolic and aqueous extracts of *Emblica offcinalis* and *Terminalia bellirica*. Activity was measured by the scavenging of DPPH radicals, and each value is expressed as the mean±standard error. There is a statistically significant difference (p≤0.001). EOA: *Emblica offcinalis* aqueous fruit extract, EOM: *Emblica offcinalis* methanolic fruit extract, TBA: *Terminalia bellirica* aqueous fruit extract, TBM: *Terminalia bellirica* methanolic fruit extract

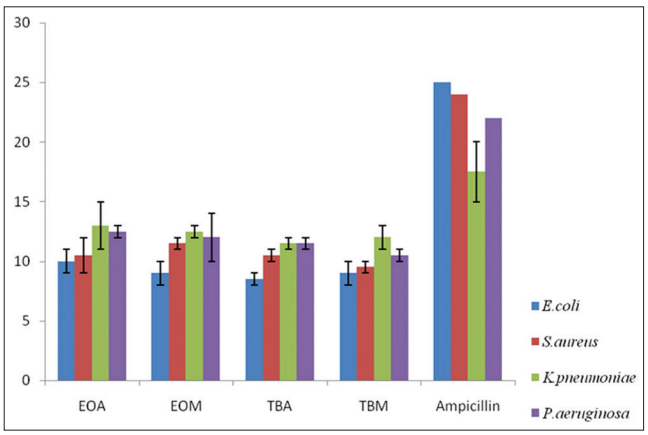

Fig. 6: Antibacterial activity. EOA: *Emblica offcinalis* aqueous fruit extract, EOM: *Emblica offcinalis* methanolic fruit extract, TBA: *Terminalia bellirica* aqueous fruit extract, TBM: *Terminalia bellirica* methanolic fruit extract, Standard: Ampicillin

Acute toxicity study

In acute toxicity study, oral administration of graded doses (5, 50, 300, 1000, and 2000 mg/kg body wt.) of the methanol extract of *E. officinalis* and *T. bellirica* to rats showed no significant changes in behavior, breathing, cutaneous effects, sensory nervous system responses, or gastrointestinal effects during the observation period. At 72 hr after administration of doses no mortality or any toxic reaction was recorded in any group. E. officinalis and T. bellirica were recorded safe up to a dose level of 2000 mg/kg body wt (Tables 2 and 3).

DISCUSSION

The phytochemical analysis and estimation of the percentage of active components of the *E. officinalis* and *T. bellirica* showed that the fruits are rich in alkaloids, flavonoids, tannins, phenols, and saponins [24]. These secondary metabolites are liable for their antibiotic activity. Flavonoids and tannins are phenolic compounds which act as primary antioxidants or scavengers of free radical [19]. DPPH antioxidant assay determined the potential of the test compounds reactivity with a stable free radical. Free radical scavenging ability of the test compound was determined by the extent of decline in absorbance measurement. Plant extracts show variability in their antimicrobial activities because of their contents of active compounds [8]. Various reports showed that alkaloids and flavonoids are the lead compounds which are liable for their antimicrobial activities [11]. While it is claimed that secondary metabolites such as tannins and some phenolic compounds are classified as active antimicrobial compounds [28]. Tannins prevent the micro-organisms by precipitating microbial protein, which act as an inhibitor for many yeasts, fungi, bacteria, and viruses [27,9].

The present study reveals that fruit extracts of *E. officinalis* and *T. bellirica* have a significant effective antimicrobial activity. The zone of inhibition formed around the wells indicates the extent of antimicrobial activity. These extracts showed an effective inhibition range against *E. coli*, *K. pneumoniae*, *S. aureus,* and *P. aeruginosa*. This is the first report on E. officinalis and T. bellirica of Dehradun, Uttarakhand region, as no toxicity study on these plants has ever been conducted. The present study indicates that *E. officinalis* and *T. bellirica* are rich sources of natural antioxidants which are very significant in disease prevention

Table 2: Acute toxicity study of *Emblica offcinalis* fruits at different dose levels

Drug treatment	Dose (mg/kg)	Signs of toxicity	Effect observed	Death
EOM	5	No signs	No adverse effect	0/3
	50	No signs	No adverse effect	0/3
	300	No signs	No adverse effect	0/3
	2000	No signs	No adverse effect	0/3

EOM: *Emblica offcinalis* methanolic fruit extract

Table 3: Acute toxicity study of *Terminalia bellirica* fruits at different dose levels

Drug treatment	Dose (mg/kg)	Signs of toxicity	Effect observed	Death
TBM	5	No signs	No adverse effect	0/3
	50	No signs	No adverse effect	0/3
	300	No signs	No adverse effect	0/3
	2000	No signs	No adverse effect	0/3

TBM: *Terminalia bellirica* methanolic fruit extract

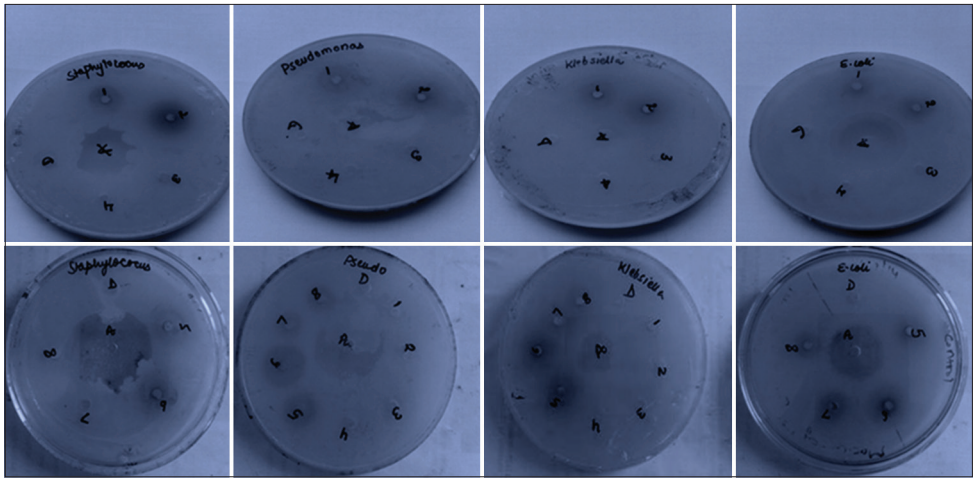

Fig. 7: Zone of inhibition. 1: EOM (*Emblica officinalis* methanolic fruit extract), 2: EOA (*Emblica officinalis* aqueous fruit extract), 5: TBM (*Terminalia bellirica* methanolic fruit extract); 6: TBA (*Terminalia bellirica* aqueous fruit extract)

and could be potent plant-derived medicines against bacteria and various complications leading to breakdown of BBB.

As no adverse changes, mortality in animals behavior and also no abnormalities were detected in experimental rats at the maximum dose of 2000 mg/kg body wt. The *E. officinalis* and *T. bellirica* fruits were found to be safe at LD_{50} dosage as per OECD recommendations.

CONCLUSION

The plant extracts used in present study exhibit the presence of various active components such as phenols, flavonoids, along with their antioxidant and antimicrobial activities which may be useful against different infections or diseases. The methanolic and aqueous fruit extracts possess effective inhibitory activity against the tested pathogens. The results of the study support the folklore claim of this plant. The antioxidant activity of plant extracts helps in reducing oxidative stress. Further pharmacological evaluations and possible isolation of the active components from these plants are major steps toward future therapeutics for various diseases. Results show that methanolic extract of *E. officinalis* and *T. bellirica* fruits do not cause any apparent *in vivo* toxicity in an animal model. No death or signs of toxicity were observed in rat treated with extract at various doses.

ACKNOWLEDGMENT

We thank Graphic Era University for their constant support during the entire course of this study.

REFERENCES

1. Al-Rehaily AJ, Al-Howiriny TA, Al-Sohaibani MO, Rafatullah S. Gastroprotective effects of 'Amla' *Emblica officinalis* on *in vivo* test models in rats. Phytomedicine 2002;9(6):515-22.
2. Aqil F, Ahmad I. Antibacterial properties of traditionally used Indian medicinal plants. Methods Find Exp Clin Pharmacol 2007;29(2):79-92.
3. Azzahra LF, Fouzia H, Mohammed L, Noureddine B. Antioxidant response of *Camellia sinensis* and *Rosmarinus officinalis* aqueous extracts toward H_2O_2 stressed mice. J Appl Pharm Sci 2012;2(7):70-6.
4. Saraphanchotiwitthaya A, Ingkaninan K. Immunomodulatory activity of an acetone extract of terminalia bellerica roxb fruit on the mouse immune response *in vitro*. Int J Pharm Pharm Sci 2014;6(11):274-8.
5. Hazra B, Biswas S, Mandal N. Antioxidant and free radical scavenging activity of *Spondias pinnata*. BMC Complement Alternat Med 2008;8:63.
6. Bloch O, Papadopoulos MC, Manley GT, Verkman AS. Aquaporin-4 gene deletion in mice increases focal edema associated with staphylococcal brain abscess. J Neurochem 2005;95:254-62.
7. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1958;181(4617):1199-200.
8. Boakye-Yiadom K, Konning GH. Incidence of antibacterial activity in the Connaraceae. Planta Med 1975;28(4):397-400.
9. Chithrashree, Murthy NK, Srinivas C. Phytochemical screening and *in vitro* assessment of antimicrobial and antioxidant potential of *Andrographis serpyllifolia* - An endemic medicinal plant from South India. Int J Adv Res 2014;2(2):917-28.
10. Deshmukh CD, Pawar AT, Bantal V. Effect of *Emblica officinalis* methanolic fruit extract on indomethacine induced enterocolitis in rats. Res J Med Plant 2010;4(3):141-8.
11. Cordell GA, Quinn-Beattie ML, Farnsworth NR. The potential of alkaloids in drug discovery. Phytother Res 2001;15(3):183-205.
12. Zheleva-Dimitrova D, Nedialkov P, Kitanov G. Radical scavenging and antioxidant activities of methanolic extracts from *Hypericum* species growing in Bulgaria. Pharmacogn Mag 2010;6(22):74-8.
13. Tang EL, Rajarajeswaran J, Fung SY, Kanthimathi MS. Antioxidant activity of *Coriandrum sativum* and protection against DNA damage and cancer cell migration. BMC Complement Altern Med 2013;13:347.
14. Jindal A, Soyal D, Sharma A, Goyal PK. Protective effect of an extract of *Emblica officinalis* against radiation-induced damage in mice. Integr Cancer Ther 2009;8(1):98-105.
15. Mallik J, Das P, Karon B, Das S. A review on phytochemistry and pharmacological activity of *Terminalia belerica*. Int J Drug Formul Res 2012;3(6):1-7.
16. Kaur G, Stetler-Stevenson M, Sebers S, Worland P, Sedlacek H, Myers C, *et al.* Growth inhibition with reversible cell cycle arrest of carcinoma cells by flavone L86-8275. J Natl Cancer Inst 1992;84(22):1736-40.
17. Todar K. The Good, the Bad, and the Deadly. SCIENCE Magazine. Vol. 304; 2004. p. 1421.
18. Cho KT, Park BJ. Gas-forming brain abscess caused by *Klebsiella pneumoniae*. J Korean Neurosurg Soc 2008;44(6):382-4.
19. Khan TH, Sultana S. Apigenin induces apoptosis in Hep G2 cells: Possible role of TNF-alpha and IFN-gamma. Toxicology 2006;217(2-3):206-12.
20. Kim HJ, Yokozawa T, Kim HY, Tohda C, Rao TP, Juneja LR. Influence of amla (*Emblica officinalis* Gaertn.) On hypercholesterolemia and lipid peroxidation in cholesterol-fed rats. J Nutr Sci Vitaminol (Tokyo) 2005;51(6):413-8.
21. Kusirisin W, Srichairatanakool S, Lerttrakarnnon P, Lailerd N, Suttajit M, Jaikang C, *et al.* Antioxidative activity, polyphenolic content and anti-glycation effect of some Thai medicinal plants traditionally used in diabetic patients. Med Chem 2009;5(2):139-47.
22. Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. BMC Complement Altern Med 2012;12:221.
23. Das N, Goshwami D, Hasan S, Raihan SZ. Evaluation of acute and subacute toxicity induced by methanol extract of *Terminalia citrina* leaves in Sprague Dawley rats. J Acute Dis 2015;4(4):316-21.
24. Singh N, Mathur C, Sase NA, Rai S, Abraham J. Pharmaceutical properties of *Emblica officinalis* and *Phyllanthus emblica* extracts. Res J Pharm Biol Chem Sci 2015;6(1):1007-16.
25. Kuppusamy P, Yusoff MM, Parine NR, Govindan N. Evaluation of *in-vitro* antioxidant and antibacterial properties of *Commelina*

nudiflora L. Extracts prepared by different polar solvents. Saudi J Biol Sci 2015;22(3):293-301.
26. Devi PN, Kaleeswari S, Poonkothai M. Antimicrobial activity and phytochemical analysis of fruit extracts of *Terminalia Bellerica.* Int J Pharm Pharm Sci 2014;6(5):639-42.
27. Prasad RN, Viswanathan S, Devi JR, Nayak V, Swetha VC, Archana BR, *et al*. Preliminary phytochemical screening and antibacterial activity of *Samanea saman*. J Med Plants Res 2008;2(10):268-70.
28. Rojas A, Hernandez L, Pereda-Miranda R, Mata R. Screening for antimicrobical of crude drug extracts and pure natural products from Mexican medicinal plants. J Ethnopharmacol 1992;35(3):275-85.
29. Sai Ram M, Neetu D, Yogesh B, Anju B, Dipti P, Pauline T, *et al.* Cyto-protective and immunomodulating properties of Amla (*Emblica officinalis*) on lymphocytes: An *in-vitro* study. J Ethnopharmacol 2002;81(1):5-10.
30. Sathya V, Bharathidasan R, Tamil Selvi S, Rebeccal NS, Ilakkiya R, Prabakaran M. Quantitative, qualitative phytochemical analysis and *in vitro* antibacterial activity of *Bauhinia tomentosa* L. J Nat Prod Plant Res 2013;3(2):31-6.
31. Firdouse S, Alam P. Phytochemical investigation of extract of *Amorphophallus campanulatus* tubers. Int J Phytomed 2011;3(1):32-5.
32. Baba SA, Malik SA. Evaluation of antioxidant and antibacterial activity of methanolic extracts of *Gentiana kurroo* royle. Saudi J Biol Sci 2014;21(5):493-8.
33. Potdar S, Lakshminarayan N, Goud RS. Antimicrobial efficacy of *Emblica officinalis* fruit extracts on *S. mutans*, *E. faecalis* and *C. albicans*. Adv Human Biol 2014;4(1):26-30.
34. Suryanarayana P, Saraswat M, Petrash JM, Reddy GB. *Emblica officinalis* and its enriched tannoids delay streptozotocin-induced diabetic cataract in rats. Mol Vis 2007;13:1291-7.
35. Sheen TR, Ebrahimi CM, Hiemstra IH, Barlow SB, Peschel A, Doran KS. Penetration of the blood-brain barrier by *Staphylococcus aureus*: Contribution of membrane anchored lipoteichoic acid. J Mol Med (Berl) 2010;88(6):633-9.
36. Magnus T, Röther J, Simova O, Meier-Cillien M, Repenthin J, Möller F, *et al.* The neurological syndrome in adults during the 2011 northern German *E. coli* serotype O104: H4 outbreak. Brain 2012;135:1850-9.
37. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft und-Technol 1995;28:25-30.
38. Yokozawa T, Kim HY, Kim HJ, Okubo T, Chu DC, Juneja LR. Amla (*Emblica officinalis* Gaertn.) Prevents dyslipidaemia and oxidative stress in the ageing process. Br J Nutr 2007;97:1187-95.
39. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid content in mulberry and their scavenging effects on superoxide radicals. Food Chem 1999;64(4):555-9.
40. Meningitis Research Foundation. Available from: http://www.meningitis.org/disease-info/types-causes/ecoli.