

Phytochemical Determination and Evaluation of Antibacterial, Antifungal and Cytotoxic Effects in Selected Medicinal Plants

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ABSTRACT

Abrus precatorious, *Terminalia arjuna* and *Terminalia chebula* are one of the most important medicinal plants used for therapeutic purpose. The present study was carried out to investigate antibacterial, antifungal and cytotoxic activity and also deals with phytochemical analysis of selected plants, *Abrus precatorious*, *Terminalia arjuna* and *Terminalia chebula*. The ethanolic extracts of seeds of all selected plants were tested for the presence of various phytoconstituents such as tannins, terpenoids, alkaloids, flavanoids, phlobatannins, cardiac glycosides and steroids. The antibacterial activity was tested against *Bacillus Subtilis* (ATCC 6633), *Streptococcus Aureus* (ATCC 6538), *Streptococcus Abony* (ATCC 6017), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027) by the agar well diffusion method. The ethanolic extracts of seeds of selected plants were tested against fungi like *Alternaria solani*, *Aspergillus niger*, *Lasiodiplodia theobromae*, *Rhizopus spp.* and *Candida Albicans* by pour plate method. The cytotoxic effect of the ethanolic extract of seeds and methanolic extract of leaves of all the plants were evaluated on c2c12 (Mouse, Muscle cell line) and HEK293T (Human embryonic kidney cell line) by MTT assay.

Key Words – Chanothi, Arjuna, Hirda, Phytochemical analysis, Antibacterial, Antifungal activity, MTT assay.

INTRODUCTION

Medicinal plants have a great significance as they contain important medicinal constituents that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs”^[1]. *Abrus precatorious* Linn (Fabaceae), ($2n=22$) is an important medicinal plant and commonly known as “Indian Liquorice, rosary pea, crab’s eye and jequirity”, in English. The major activity of the plant seeds till now reported are antitumour, antiinflammatory, antiallergic, insecticidal, antibacterial, antidiarrhoeal^[2]. *Terminalia arjuna* (Combretaceae), ($2n=24$) is a large tree and commonly known as Arjuna in India^[3]. It is a good cardio protective, hepatoprotective, tonic, anticoagulant, antihypertensive, antiviral, antifungal and antibacterial agent^[4]. *Terminalia chebula* (Combretaceae), ($2n=72$) is one of the most important medicinal plants and also known as black myrobalan; a king of medicine or hirda^[5]. It is used for the treatment of number of diseases like cancer, paralysis, cardio vascular diseases, ulcers, arthritis, etc. and also reported as antioxidant, antidiabetic, antibacterial, antiviral, antifungal, anticancerous, antimutagenic, etc^[6].

The study has been undertaken to screen phytochemical components of *Abrus precatorious*, *Terminalia arjuna* and *Terminalia chebula* seeds. We have also evaluated their antibacterial potential against *B. Subtilis*, *S. Aureus*, *S. Abony*, *E. coli*, *P. aeruginosa* and the antifungal effects against *Aspergillus niger*, *Alterneria solani*, *Rhizopus spp.* and *Lasioidiplodia theobromeae*. The present study was designed to find out the cytotoxic activity of the selected plants using different concentrations of extracts against c2c12 (Mouse, Muscle cell line) and HEK293T (Human embryonic kidney cell line) using the MTT assay.

MATERIALS AND METHODS

Plant material collection: The plant materials used in this study were leaves and seeds of *Abrus precatorious* collected from herbal farm Chakalasi and Bedwa. Seeds and leaves of *Terminalia arjuna* and seeds of *Terminalia chebula* which were collected and all collected material was identified from Directorate of Medicinal and Aromatic Plants Research Center at Boriavi, Gujarat. The seed and leaves were air dried and ground well into a fine powder. The powder was stored in air sealed polythene bags at room temperature before extraction.

Extraction protocol: For extraction, about 20 gm of the dried and powdered plant material (seeds and leaves) was taken and extracted with 50 ml solvent (70 % ethanol and methanol). The mixture was kept for 48-72 hrs in a rotary shaker at room temperature. All extracts were filtered individually through Whatman No.1 filter paper and filtrate is directly used for phytochemical analysis^[7]. For other analysis, filtrate was poured in petridishes and kept for evaporation of the liquid solvents. After drying, crude extracts were dissolved in DMSO (Dimethyl sulfoxide) and then used for antibacterial, antifungal and cytotoxic activity.

Phytochemical analysis: The 70% ethanolic extracts of seeds of all three selected plants were tested for the presence of various phytoconstituents such as tannins, terpenoids, alkaloids, flavanoids, phlobatannins, cardiac glycosides and steroids by following tests ^[7,8] :

Test for Tannin: Few drops of FeCl₃ solution were added to filtrate. Appearance of brownish green or deep black blue colour indicated the presence of tannin.

Test for Terpenoids: Filtrate was mixed with chloroform and concentrated H₂SO₄ was carefully added to form layer. A reddish brown colour or ring formed indicates the presence of terpenoids.

Test for Alkaloids: Filtrate was treated with Hager's reagent (saturated picric acid solution) Formation of yellow colored precipitate indicates the presence of alkaloids.

Test for Flavonoids: Few drops of lead acetate solution were added in filtrate. Formation of yellow colour or precipitate indicates the presence of flavonoids.

Test for Phlobatannin: Aqueous HCL was added in filtrate and boiled. Formation of red colour or precipitate indicates the presence of phlobatannin.

Test for Cardiac Glycoside: Glacial acetic acid was added to filtrate followed by addition of few drops of FeCl₃ solution and concentrated H₂SO₄. Development of brown colour ring indicates the presence of cardiac glycosides.

Test for Steroids: The filtrate was treated with few drops of concentrated H₂SO₄ and shaken. Formation of red colour indicates the presence of steroids.

Antibacterial activity: Ethanolic extract of *Abrus precatorious*, *Terminalia arjuna* and *Terminalia chebula* seeds were concentrated and finally dissolved in DMSO and used for testing antibacterial activity. The microbial strains used in this study were *Bacillus Subtilis* (ATCC 6633), *Streptococcus Aureus* (ATCC 6538), *Streptococcus Abony* (ATCC 6017), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027)^[9]. The cultures were stored

Table-1: Phytochemical analysis of *Abrus precatorious*, *Terminalia arjuna* & *Terminalia chebula*.

No. (PC)	Test	<i>Abrus precatorious</i>	<i>Terminalia arjuna</i>	<i>Terminalia chebula</i>
1	Tannins	+ve	+ve	+ve
2	Terpenoids	+ve	+ve	+ve
3	Alkaloids	+/-ve	+ve	+ve
4	Flavonoids	+ve	+ve	+ve
5	Phlobatannins	+ve	-ve	-ve
6	Cardiac glycosides	+ve	+ve	+ve
7	Steroids	+ve	+ve	+ve

+ve = Phytochemical Present, -ve = Phytochemical Absent, +/- = Either Phytochemical present or absent

Table-2 Antibacterial activity of *Abrus precatorious*, *Terminalia arjuna* & *Terminalia chebula*.

Test microorganisms	<i>Abrus precatorious</i>	<i>Terminalia arjuna</i>	<i>Terminalia chebula</i>
<i>Bacillus subtilis</i>	32mm	10mm	24mm
<i>Staphylococcus aureus</i>	8mm	6mm	10mm
<i>Staphylococcus abony</i>	10mm	16mm	8mm
<i>Escherichia coli</i>	26mm	6mm	16mm
<i>Pseudomonas aeruginosa</i>	6mm	6mm	14mm

on nutrient agar slants at 4° C. Antibacterial activity of the test extracts was tested by agar well diffusion method using molten nutrient agar^[10]. After solidification of the medium, wells were made and test extracts were added into the wells, separately. Plates were incubated at 37°C for 24 h. The antibacterial activity of the test samples was determined by measuring the diameter of clear zone around the well.

Antifungal activity: Ethanolic extract of *Abrus precatorious*, *Terminalia arjuna* and *Terminalia chebula* seeds were concentrated and dissolved in DMSO and used for testing antifungal activity. The fungi used were *Alternaria solani*, *Aspergillus niger*, *Lasiodiplodia theobromae*, *Rhizopus* and *Candida Albicans*. These were most common and important disease causing fungi of plants and seeds of the region. All these fungi were obtained from Anand Agriculture University and pure cultures were maintained on PDA^[11]. The fungal spores were inoculated in PDB allowed it grow for 24 to 48 hrs. Then these spores were transferred to autoclaved PDA media, mixed properly and poured in sterile petri plate and allowed to solidify^[12]. Then wells were made and test extracts were added into the wells. Plates were incubated at 28° C for 24 to 48 hrs. The antifungal activity of the test samples was determined by measuring the diameter of clear zone around the well.

MTT cytotoxicity assay-

Preparation of plant extract: Ethanolic extract of seeds and methanolic extract of leaves of *Abrus precatorious*, *Terminalia arjuna* and *Terminalia chebula* were prepared using soxhlet apparatus method. All the extracts were poured into sterile dry petriplates and the organic solvents were evaporated. The sediments were scrapped off, dissolved in DMSO and three concentrations, low dose (50ug/ml), mid dosage (250ug/ml), and high dosage (500ug/ml) were prepared from that and used along with DMSO as a negative control for all the plants used in the study.

Figure-1

Figure-2

Figure-3



C = Chanothi
(*Abrus precatorious*)

A = Arjuna
(*Terminalia arjuna*)

H = Hirda
(*Terminalia chebula*)

Table-3 Antifungal activity of *Abrus precatorious*, *Terminalia arjuna* & *Terminalia chebula*.

	<i>Abrus precatorious</i>	<i>Terminalia arjuna</i>	<i>Terminalia chebula</i>
<i>Candida albicans</i>	8mm	26mm	28mm
<i>Aspergillus niger</i>	17mm	13mm	25mm
<i>Alternaria solani</i>	30mm	NF	NF
<i>Lasiodiplodia thebromeae</i>	15mm	17mm	18mm
<i>Rhizopus</i>	23mm	20mm	35mm

NF=NOT FOUND

Cell-lines used: The cytotoxic activity of the extract was evaluated on c2c12,(Mouse, Muscle cell line)^[13] and HEK293T (Human embryonic kidney cell line) cell lines which were procured as a kind gift from Dr. C.G. Joshi, Anand Agriculture University, Anand. Both the cell lines were grown in DMEM supplemented with 10% foetal bovine serum (FBS) and 1% penicillin, streptomycin, neomycin (PSN) at 37°C in a 5% CO₂, 95% humidified atmosphere.

Assay protocol: Principle of the test is to convert the yellow tetrazolium salt MTT to purple formazan crystals by metabolically active cells. The amount of formazan produced is proportional to the number of viable cells. Cells were plated in 96-well flat bottom tissue culture plates at a density of approximately 10,000 cells/well and allowed to attach overnight at 37°C. The cells were then incubated with the extract at a concentration of 50µg/mL, 250 µg/mL and 500µg/mL for 24 hours. Untreated cultures and blank wells without cells received negative control for respective controls. After the drug exposure period, the cells were grown for an additional 24 hours in extract-free fresh medium. Next, 20 µL of the MTT (5mg/ml) reagent was added to each well, and the plate was incubated for 4 hours at 37°C. The MTT crystals were then solubilized in 200 µl of DMSO. Absorbance measurements were made at 570 nm using a Biotek ELISA plate reader. Proliferation was expressed as the fraction of treated cells that survived relative to untreated cultures. Every experiment included a set of negative controls (untreated cultures). All experiments were performed in triplicate^[14].

The percentage of cytotoxicity was calculated using the following formula^[15]:

$$\% \text{ Cytotoxicity} = (1 - \text{Abs test} / \text{Abs Control}) \times 100.$$

RESULTS

Phytochemical analysis: The phytochemical screening of the plants studied showed the presence of tannins, terpenoids, alkaloids, flavanoids, cardiac glycosides and steroids in ethanolic extracts of seeds of *Abrus precatorious*, *Terminalia arjuna* and *Terminalia chebula*. Both *Terminalia arjuna* and *Terminalia chebula* showed the absence of phlobatannins whereas *Abrus precatorious* showed its presence (Table- 1).

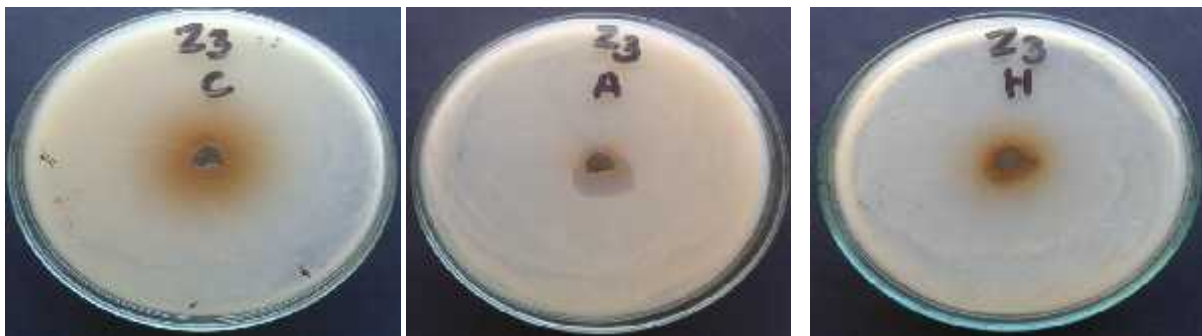
Z1 (*Bacillus subtilis*)



Z2 (*Staphylococcus aureus*)



Z3 (*Staphylococcus abony*)



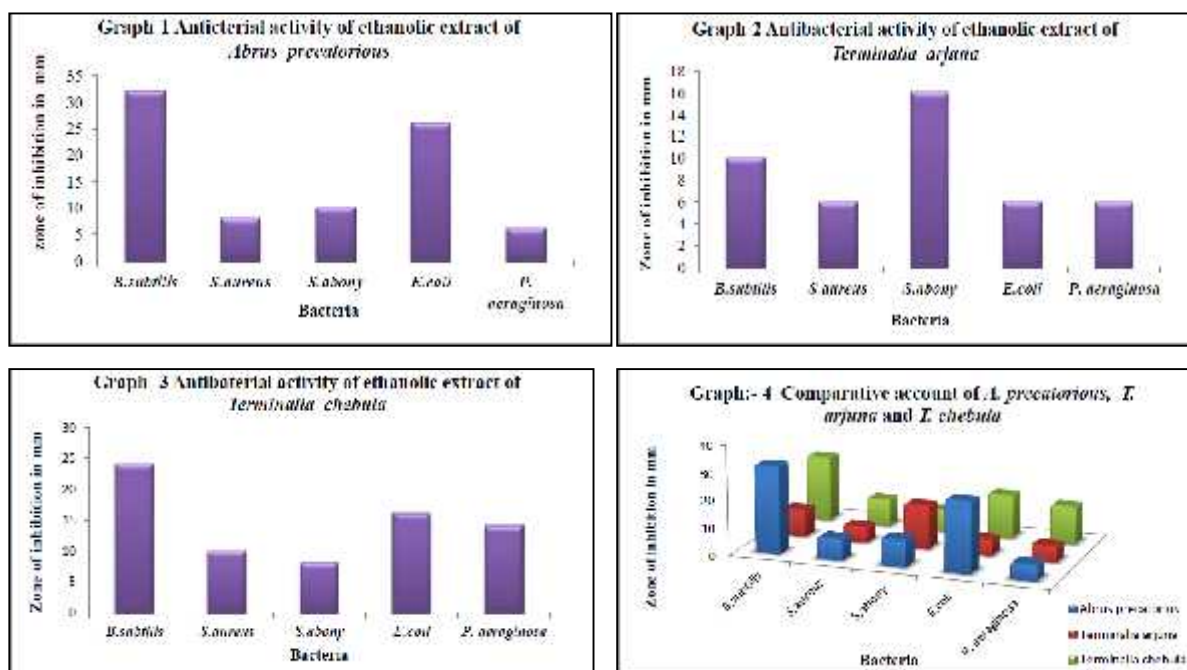
Z4 (*Escherichia coli*)



Z5 (*Pseudomonas aeruginosa*)



C = *Abrus precatorious*, A = *Terminalia arjuna*, H = *Terminalia chebula*



Antibacterial activity: The ethanolic extract of the seeds of *Abrus precatorious*, *Terminalia arjuna* and *Terminalia chebula* were tested for the antibacterial activity against a number of Gram positive and Gram negative bacteria. Ethanolic extract of *Abrus precatorious* seeds showed the highest activity against *Bacillus subtilis* followed by *E. coli*, *Staphylococcus abony* and moderate activity against *Staphylococcus aureus* followed by *Pseudomonas aeruginosa*.(graph-1) Ethanolic extract of *Terminalia arjuna* seeds showed the highest activity against *Staphylococcus abony* and *Bacillus subtilis* but moderate activity against *Staphylococcus abony*, *E.coli* and *Pseudomonas aeruginosa*.(graph-2) Ethanolic extract of *Terminalia chebula* seeds showed the highest activity against *Bacillus subtilis* followed by *E.coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* but only *Staphylococcus abony* showed moderate activity. (graph-3)

Antifungal activity: The ethanolic extract of the seeds of *A. precatorious*, *T. arjuna* and *T. chebula* were tested for the antifungal activity against the many fungi *C. albicans*, *A. niger*, *A. solani*, *L. theobromeae* and *Rhizopus spp.*

Ethanolic extract of *Abrus precatorious* seeds showed the highest activity against *Alterneria solani* followed by *Rhizopus spp.*, *Aspergillus niger*, *Lasiodiplodia thebromeae* and moderate activity against *Candida albicans*. (graph-5) Ethanolic extract of *Terminalia arjuna* seeds showed the highest activity against *Candida albicans*

Table-4: Cytotoxic properties of *Abrus precatorious*, *Terminalia arjuna* & *Terminalia chebula* on different cell line.

Plant Name	Plant part	Solvent	Concentration (ug/ml)	% Cytotoxicity	
				HEK293T	c2c12
<i>Abrus precatorious</i>	Seeds	Ethanol	50	-37.27 ± 0.18	153.90 ± 0.020
			250	2.72 ± 0.02	212.84 ± 0.003
			500	27.27 ± 0.06	172.90 ± 0.035
	Leaves	Methanol	50	75.28 ± 0.02	215.92 ± 0.007
			250	104.49 ± 0.01	241.62 ± 0.004
			500	103.37 ± 0.01	251.11 ± 0.010
<i>Terminalia arjuna</i>	Seeds	Ethanol	50	8.98 ± 0.18	148.88 ± 0.052
			250	17.97 ± 0.02	172.62 ± 0.008
			500	10.11 ± 0.06	NR
	Leaves	Methanol	50	-71.81 ± 0.16	125.13 ± 0.037
			250	NR	- 100.55 ± 0.016
			500	NR	-28.21 ± 0.037
<i>Terminalia chebula</i>	Seeds	Ethanol	50	-23.63 ± 0.16	161.73 ± 0.035
			250	-10.00 ± 0.02	-4.46 ± 0.043
			500	4.54 ± 0.01	49.72 ± 0.002

Results summarized here are the mean values from three parallel experiments ± S.D.

NR = No reaction under experimental condition

No activity was reported in *Terminalia chebula* leaves

followed by *Rhizopus spp.*, *Lasiodiplodia thebromae* and *Aspergillus niger* but no activity against *Alternaria solani*. (graph-6). Ethanolic extract of *Terminalia chebula* seeds showed the highest activity against *Rhizopus spp.* followed by *Candida albicans*, *Aspergillus niger* and *Lasiodiplodia thebromae* but no activity against *Alternaria solani*. (graph-7)

Cytotoxicity assay : The results of the cytotoxicity against selected cancer cell lines are shown below (Table 4). The cytotoxic activity result showed that seed extract of *Abrus precatorious* exhibited better cytotoxic effects on c2c12 (212.84 ± 0.003) and HEK293T (27.27 ± 0.06) followed by *Terminalia arjuna* on c2c12 (172.62 ± 0.008) and HEK293T (17.97 ± 0.02). *Terminalia chebula* expressed comparatively lower activities on c2c12 (161.73 ± 0.035) and HEK293T (4.54 ± 0.01). The cytotoxic activity result showed that leaf extract of *Abrus precatorious* exhibited better cytotoxic effects on c2c12 (251.11 ± 0.010) and HEK293T (104.49 ± 0.01) followed by *Terminalia arjuna* expressed comparatively lower activities on c2c12 (125.13 ± 0.037) and HEK293T (-71.81 ± 0.16).

Cytotoxicity results showed that both seed and leaf extracts of *Abrus precatorious* exhibited comparatively higher cytotoxic effects on c2c12 and HEK293T line while the efficacy was lower in *Terminalia arjuna* and *Terminalia chebula* after 24 hrs incubation.

DISCUSSION

A.N (Aspergillus niger)



C (Candida albicans)



A (Altenaria solani)

A = *T.arjuna*

H = *T. chebula*, C = *A. precatorious*

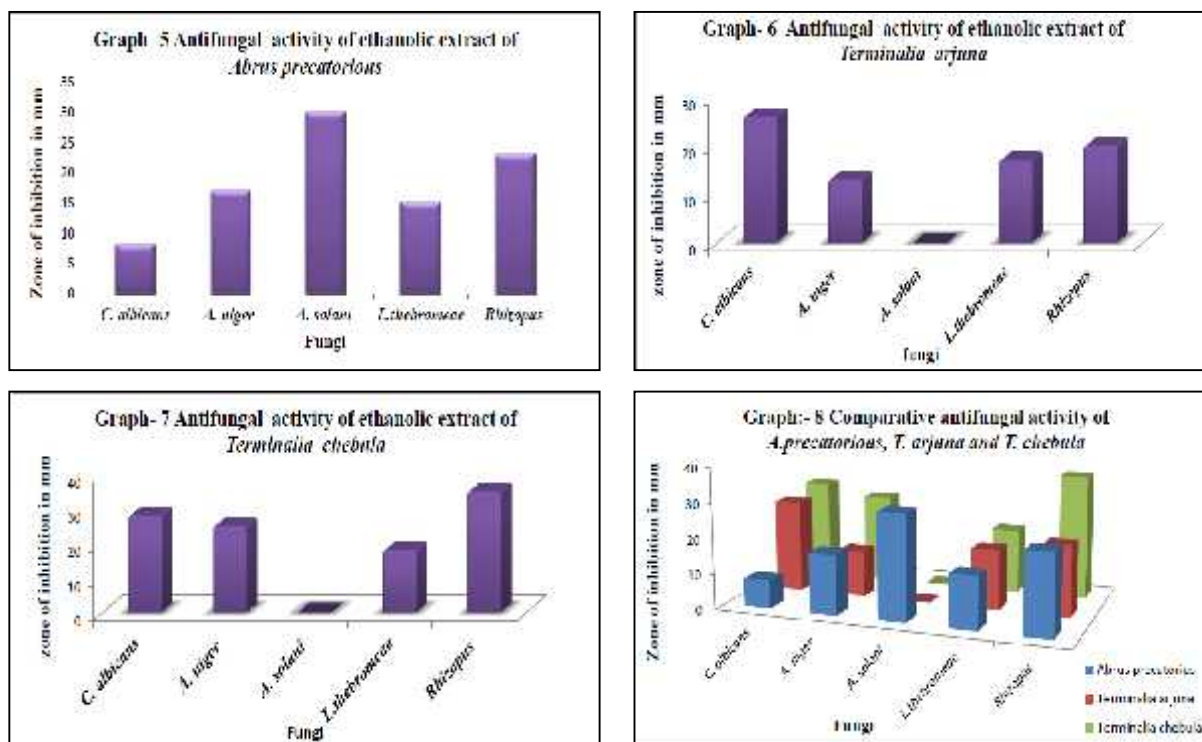
A = *Terminalia arjun*

L (Lasiodiplodia thebromeae)



R (Rhizopus)





The selected plants showed presence of all phytochemicals (tannins, flavonoids, terpenoids, cardiac glycosides, steroids, alkaloids) except phlobatannin, which was present only in *Abrus precatorious*.

Seed extracts of *A. precatorious* showed highest activity against *Bacillus subtilis* with zone diameter of 32 mm followed by *Escherichia coli* (26 mm). Seeds extract of *T. arjuna* showed highest zone of inhibition against *Staphylococcus abony* with zone diameter (16mm) followed by *Bacillus subtilis* (10 mm). *T. chebula* seeds extract showed highest zone of inhibition against *Bacillus subtilis* with zone diameter (24mm) then followed by *Escherichia coli* with zone diameter (16 mm). The results obtained are in agreement with the Previous studies on all three plants selected^[16,17,18,19]. However, there haven't been any reports on three plants inhibiting *S. abony*, which was reported in this work.

All the plants also showed good activity against selected fungi. Significant activities were seen in seed extracts of *T. chebula* against *Rhizopus* with zone diameter (35 mm) and *Candida albicans* with zone diameter (28 mm) followed by seeds extract of *A. precatorious* against *Alterneria solani* with zone diameter (30 mm) and *Rhizopus* (23mm) and then by seeds extract of *T. arjuna* against *Candida albicans* with zone diameter (26mm) and *Rhizopus* with zone diameter (20 mm).The results show that both *Terminalia* sps. have antifungal activity against all fungi tested except *Alternaria*.

Cytotoxicity results showed that leaf extracts of *Abrus precatorious* exhibited comparatively higher cytotoxic effects on c2c12 (251.11 ± 0.010) and HEK293T (104.49 ± 0.01) line followed by seed extracts of *Abrus precatorious* on c2c12 (212.84 ± 0.003) and HEK293T (27.27 ± 0.06). *Terminalia arjuna* expressed comparatively lower activities on c2c12 (172.62 ± 0.008) and HEK293T (17.97 ± 0.02) and *Terminalia chebula* on c2c12 (161.73 ± 0.035) and HEK293T (4.54 ± 0.01) while the efficacy was lower in leaf extracts of *Terminalia arjuna* on c2c12 (125.13 ± 0.037) and HEK293T (-71.81 ± 0.16). Phytochemicals such as terpenoids, flavonoids,

alkaloids, tannins, enzymes and minerals have been found to elicit anticancer activities. These chemicals block various hormone actions and metabolic pathways that are associated with the development of cancer [20,21].

CONCLUSIONS

From the above investigation it can be concluded that the extract of above mentioned medicinal plants can be considered as a valuable resource for potential antibacterial, antifungal and anticancer agents. The present results therefore offer a scientific basis for traditional use of the extracts of *Abrus precatorious* and both *Terminalia* spp. could be a possible source to obtain new and effective herbal medicines to treat infections caused by multi-drug resistant strains of microorganisms. Therefore, there is an urgent need to investigate the biological activity of its phytoconstituents for development of an effective, safe and cheap herbal drug.

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