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In vitro antibacterial study of aqueous and methanolic extracts of some selected medicinal plants

Sachin Kumar, Hotam Singh Choudhary, Chandrabhan Seniya*

Department of Biotechnology, Madhav Institute of Technology and Science, Gwalior, M. P., India

ABSTRACT

In the present study six aqueous and methanolic plant extracts (Adhatoda vasica, Nyctanthes arbortristis, Phyllanthus amarus, Vitex Negundo, Terminalia arjuna and Terminalia chebula) from botanical species have been subjected to a screening study to detect potential antibacterial activity against Staphylococcus aureus and Escherichia coli. The antibacterial activity of the products was evaluated using colonies growing in solid medium, establishing the zone of inhibition in vitro growth (ZOI). A small portion of the dry extract was used for the phytochemical tests for compounds which include alkaloids, hydrolysable tannins, flavonoids, saponins and glycosides in accordance with the methods. The results showed that extracts from Terminalia arjuna and Terminalia chebula possess strong in vitro antibacterial activity against the tested microorganisms.

Keywords: Plant Extracts Aqueous Extracts, Methanolic Extracts, Phytochemical.

INTRODUCTION

Plants are major source of herbal medicines and the presence of secondary metabolites in plants implicated them for many therapeutic activities [1,2]. Also, the plants had provided a source of inspiration for novel drug compounds, as plants derived medicines had made large contributions to human health and well being. A major part of the total population in developing countries still uses traditional folk medicine obtained from plant resources [3]**.** The people of Indian villages used crude plants as medicine since Vedic period and still continued.

Worldwide, the infectious diseases caused the death of approximately one-half of all deaths in tropical countries. Perhaps the infectious diseases statistics should be very high and frequent in developing nations, but it might be remarkable that infectious diseases mortality rates were actually increasing in developed countries [3]**.**

Now a days, the herbal drugs popularity increased and used widespread. The research is still lagging behind to get the efficacy of plant derived medicines on microorganisms which induced pathogenesis in Human beings and other animals. There were reports of various medicinal plants that had been used for years in daily life to treat disease all over the world. There were several reports on the antimicrobial activity of different herbal extracts in different regions of the world [4]. Phytomedicines derived from plants have shown great promise in the treatment of intractable infectious diseases [5]. A special feature of higher plants is their capacity to produce a large number of secondary metabolites [6].

The most important bioactive constituents of these plants are alkaloids, tannins, flavonoids and phenolic compounds [7]. Stuffness and Douros [8] reported that more than 50% of all modern clinical drugs were origin from natural products. The natural products might be played an important role in drug development programs in the pharmaceutical industry [9]. It had been reported that aqueous and methanolic extracts from plants used in allopathic medicines were potential sources of antiviral, antitumoral and antimicrobial agents [10]. The Higher plants, as a source of medicinal compounds, had continued to play a dominant role in the maintenance of human health since ancient times [11].

There was another aspect of drugs used against the microorganisms. The multiple drugs used against the microorganism had developed resistance due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease [12]. There were reports on the alarming incidence of antibiotic resistance in bacteria of medical importance [13]. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host, including hypersensitivity, immune suppression and allergic reactions [14].

This situation forced scientists to search for new antimicrobial substances from different plant sources. The natural products derived from plants were being tested for presence of new drugs with new modes of pharmacological action. Also, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants or a constant need for new and effective therapeutic agents [15,16]. Recent studies were involved in the identification and isolation of new therapeutic compounds of medicinal importance from higher plants for specific diseases [17,18].

In the present experiment, the eight different medicinal plants were used for phytochemical screening and to study their antibacterial efficacy (Table 1). The antimicrobial activity in *in vitro* of these plants part extract in aqueous and methanol (10% and 15%) were tested against multi drug resistance strain of *E.coli* and *S. aureus* that causes the most common cases of infectious diseases.

EXPERIMENTAL SECTION

Collection of medicinal plants

The plant leaves, roots, root bark and fruits were collected from Sanjivini City Centre, Gwalior. They were further identified for physical characteristics of flower, leaf, root, and fruit morphology in Department of Botany, Jiwaji University, Gwalior (India).

Plant parts powder and extract preparation

5gm and 7.5gm of the plant parts powder was dissolved in 45 ml of solvent (water and methanol) to prepare 10% and 15% extract in a 100 ml flask. The flask was covered with the aluminum foil and kept on rotating shaker (500 rpm) for 2 days. The solution was filtered twice, firstly with cheese cloth (four fold) and then with Whatman's filters paper. The filtrates were collected in Falcon tubes and were concentrated upto dryness by keeping it in incubator at 35ºC**.** The concentrated filterate was further dissolved in distilled water to the concentration of 10 mg/ml for 10% and 15 mg/ml for 15% extract.

Bacterial culture preparation and determination of Zone of inhibition (ZOI)

Multi Drug Resistant (MDR) Strains of *S. aureus* and *E. coli* were collected from DRDE, Gwalior. The strains were grown in Nutrient broth by keeping in incubator for 24 hrs at 37^0C.
Muller Hinton Agar (200ml) was prepared and poured into sterile petriplates. Six Petriplates were prepared for each bacterial culture; in which both types of solvents water and methanol, at two different concentrations (10% and 15%) were added for *Staphylococcus aureus and Escherichia coli*. The total 12 petriplates were inoculated with bacterial cultures. The circular wells were prepared in the agar plate with the help of micro tip (diameter of 6mm) and 50 µl of each plant extract was added in agar well with the help of micropipette. The petriplates were inoculated and kept in incubator at 37^0C for 24 hrs. The inoculated petriplates with *E. coli* and *S. aurues* in plant parts extract was observed after 24 hrs.

Phytochemical Analysis

Specific qualitative tests were performed for the presence or absence of phyto-chemicals *viz.*, alkaloids, hydrolysable tannins, flavonoids, saponins and glycosides in leaves, bark, root and fruit extract to identify the constituents by the methods described by Trease and Evans [19].

Test for Alkaloids (Mayer's test): 2.0ml of extract was measured in a test tube to which picric acid solution was added. The formation of orange coloration indicated the presence of alkaloids
Test for hydrolysable tannins: 4 ml of the extract was shaken in a test tube, after which 4ml of 10% ammonia solution was added. Formation of an emulsion on shaking indicated the presence of hydrolysable tannins.

Test for Glycosides: 25ml of dilute sulphuric acid was added to 5ml of extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10% NaOH, and then 5ml of fehling solution A and B was added. A brick red precipitate of reducing sugars indicates presence of glycosides.

Test for Saponins: Froth test for saponins was used. 1g of the sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5 min. The mixture was filtered and 2.5ml of the filtrate was added to 10ml of sterile distilled water in a test

tube. The test tube was stopped and shaken vigorously for about 30 second. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

Test for Flavonoids: 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant part extract followed by addition of concentrated H_2SO_4. Formation of yellow color observed in each extract indicated the presence of flavonoids.

RESULTS AND DISCUSSION

10% aqueous extracts of 6 medicinal plants *Adhatoda vasica, Nyctanthes arbortristis, Phyllanthus amarus, Vitex Negundo, Terminalia arjuna* and *Terminalia chebula* contains antibacterial bioactive compounds; which inhibited the growth of the *E. coli* while the growth of *S. aureus* was inhibited by *Terminalia arjuna,* and *Terminalia chebula*. The aqueous extract of *Terminalia chebula* contains more bioactive compounds; as it gave the maximum inhibitory effect on both strains.

The study was also performed on 15% aqueous extracts of 6 medicinal plants. The plant *Terminalia arjuna* inhibited the growth of *E. coli* and *S. aureus*. The diameter of ZOI was measured around the well in the petriplates. The areas away from the well contain the bacterial colonies was observed through inverted microscope. ZOI for each concentrated plant extract against each bacterial strain was measured with the help of scale and tabulated (Table 1-4). The well diameter (i.e., 6mm) was subtracted when tabulated to calculate ZOI. The maximum ZOI was recorded for *Terminalia chebula* on *S. aereus* (10mm). Therefore, it is concluded that *Terminalia chebula* is the only plant which shows maximum inhibitory effect for the aqueous extracts of both concentrations (10 % and 15%) on *E. coli* (ZOI 9 mm) and *S. aereus* (ZOI 10 mm). The experiments on 10% and 15% methanolic extracts were also performed. *Terminalia chebula* found very sensitive to both strains *E. coli* and *S. aereus*; with ZOI 10 and 12mm respectively (Table 3). To get more accurate inhibition effect of methanolic extract the 15% concentration extract was used. From this investigation an interesting results was found. *Terminalia chebula* again inhibiting the growth of both strains *E. coli* and *S. aereus* with ZOI 12 and 13mm respectively (Table 4). The inhibition of the growth of the test strains is due to the available bioactive and therapeutic compounds.

Table 1: Antibacterial activity of 10% aqueous extracts on *E. coli* and *S. aereus*

S. No	Name of Medicinal Plants	*E. coli*		*S. aureus*	
		Activity	ZOI (mm)	Activity	ZOI(mm)
1.	*Adhatoda vasica*	R	-	R	-
2.	*Nyctanthes arbortristis*	R	-	R	-
3.	*Phyllanthus amarus*	R	-	R	-
4.	*Terminalia arjuna*	S	8	S	8
5.	*Terminalia chebula*	S	10	S	9
6.	*Vitex Negundo*	R	-	R	-

Phytochemical analysis of leaves, bark, roots and fruit extract revealed the presence of or absence of phyto-chemicals viz., alkaloids, hydrolysable tannins, flavonoids, saponins and glycosides (Table5, 6). Tannins have been found to form irreversible complexes with proline rich

proteins resulting in the inhibition of the cell protein synthesis, play an important role of potent antioxidant [19].

Table 2: Antibacterial activity of 15% aqueous extracts on *E. coli* and *S. aereus*

S. No	Name of Medicinal Plants	*E. coli*		*S. aureus*	
		Activity	ZOI(mm)	Activity	ZOI(mm)
1.	*Adhatoda vasica*	R	-	R	-
2.	*Nyctanthes arbortristis*	R	-	R	-
3.	*Phyllanthus amarus*	R	-	R	-
4.	*Terminalia arjuna*	S	9	S	8
5.	*Terminalia chebula*	S	11	S	10
6.	*Vitex Negundo*	R	-	R	-

Table 3: Antibacterial activity of 10% Methanolic extracts on *E. coli* and *S. aereus*

S. No	Name of Medicinal Plants	*E. coli*		*S. aureus*	
		Activity	ZOI(mm)	Activity	ZOI(mm)
1.	*Adhatoda vasica*	R	-	S	7
2.	*Nyctanthes arbortristis*	R	-	S	6
3.	*Phyllanthus amarus*	R	-	S	6
4.	*Terminalia arjuna*	S	7	S	10
5.	*Terminalia chebula*	S	10	S	12
6.	*Vitex Negundo*				

Table 4: Antibacterial activity of 15% Methanolic extracts on *E. coli* and *S. aureus*

S. No	Name of Medicinal Plants	*E. coli*		*S. aureus*	
		Activity	ZOI(mm)	Activity	ZOI(mm)
1.	*Adhatoda vasica*	R	-	S	5
2.	*Nyctanthes arbortristis*	S	2	S	6
3.	*Phyllanthus amarus*	R	-	S	5
4.	*Terminalia arjuna*	S	9	S	11
5.	*Terminalia chebula*	S	12	S	13
6.	*Vitex Negundo*	S	1	S	7

Table 5: Phytochemical screening of aqueous extracts of six medicinal plants

Species → Tests ↓	***Phyllanthus amarus***	***Adhatoda vasica***	***Terminalia arjuna***	***Terminalia chebula***	***Nyctanthes arbortristis***	***Vitex Negundo***
Alkaloids	+	+	+	+	+	+
Hydrolysable Tannins	+	+	+	+	-	+
Flavonoids	+	-	+	+	+	+
Saponins	+	+	+	+	-	-
Glycosides	-	+	+	+	+	-

Where, + Presence of bioactive compounds; - Absence of bioactive compounds

From this analysis it might be concluded that the bark and fruit extracts of plants *Terminalia arjuna,* and *Terminalia chebula* could be used for the treatment of the diseases produced by *S.*

aureus and *E. coli* as these strains became multi drug resistant (MDR) and also for other MDR species.

Table 6: Phytochemical screening of methanolic extracts of six medicinal plants

Species → / Tests ↓	*Phyllanthus amarus*	*Adhatoda vasica*	*Terminalia arjuna*	*Terminalia chebula*	*Nyctanthes arbortristis*	*Vitex Negundo*
Alkaloids	+	+	+	+	+	+
Hydrolysable Tannins	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+

Where, + Presence of bioactive compounds; - Absence of bioactive compounds

CONCLUSION

The phytochemical analysis revealed the bioactive compounds which are responsible for the In vitro antibacterial of *Terminalia arjuna*, and *Terminalia chebula* over multi drug resistant bacteria (i.e. *E. coli* and *S. aerues*) in aqueous and methanolic extracts could be alkaloids, tannins, hydrolysable tannins, flavonoids, saponins, terpenoids, glycosides, cardic glycosides, and steroids.

The Zone of Inhibition (ZOI) was recorded and minimum inhibitory concentrations (MIC) were determined by well and disc diffusion methods. From this investigation an interesting results was found that *Terminalia chebula* inhibited the growth of both strains *E. coli* and *S. aereus* with highest ZOI 12mm and 13mm respectively with 15% methanolic and aqueous extracts. *Terminalia chebula* was also found active with all concentrations for aqueous and methanolic preprations.

From this analysis it might be concluded that the bark and fruit extracts *of Terminalia arjuna* and *Terminalia chebula* could be used for the treatment of diseases caused by *S. aureus* (such as stye, boil, abscess (faruncle, carbuncle), wound infections, cellulitis, impetigo, septicaemia metastatic abscesses, endocarditis, toxic shock syndrome, food poisoning, scalded skin syndrome, metastatic abscesses (almost anywhere)) and by *E. coli* (such as food poisoining, diarrhea, gastroenteritis, urinary tract infections, and neonatal meningitis, haemolytic-uremic syndrome, peritonitis, mastitis, septicaemia and Gram-negative pneumonia).

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