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Evaluation of total phenols and antibacterial activity of certain drug plants against some bacterial species

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Abstract : The present paper deals with antimicrobial activity of *Glycyrrhiza glabra, Tinospora cordifolia, Tribulus terrestris, Thevetia peruviana, Adhatoda vasica, Vitex negungo, Apium graveolens* and, *Annona squamosa*. It was studied against *Bacillus cereus, Staphylococcus aureus, Salmonella typhy B (STB), Shigella dysenteriae* using solvent (methanol and petroleum ether) extraction method. Methanol extract of all eight plants showed prominent inhibitory activity against *S. dysenteriae* and *STB* while *G. glabra, V. negundo*, and *T. peruviana* showed maximum zone of inhibition against four studied bacterial species. These results suggest that methanol extract is more effective than petroleum ether for the antimicrobial activity. Further total phenols were estimated and antibacterial activity of these plants in relation to total phenolic content is discussed.

Keywords: Antibacterial activity, Methanol extract, Petroleum ether extract, Total phenols

INTRODUCTION

The use of the plants, plant extracts and pure compounds isolated from natural sources provided the foundation to modern pharmaceutical compounds. The interest in the study of medicinal plants as a source of pharmacologically active compounds has increased worldwide. Several studies indicated that medicinal plants contain substances like peptide, unsaturated long chain fatty acids, aldehydes, alkaloids, essential oils, phenols and water or ethanol soluble compounds. These compounds are potentially significant in therapeutic applications against human and animal pathogens, including bacteria, fungi and viruses (Iwu *et al*., 1999 and Khan *et al*., 2003).

It is recognized that, in some developing countries plants are the main medicinal source to treat infectious diseases (Al-Bayati and Al-Mola, 2008). Contrary to synthetic drugs, antimicrobials of plant origin usually are not associated with many side effects and have an enormous anti-infective potential in numerous infectious diseases. Detailed research on the chemistry and pharmacology of products of plant origin are much essential and this may eventually lead to the discovery of medicine that can be used in the treatment of several diseases. Studies can show that the toxic effects of radiations and chemotherapy in cancer treatment could be reduced by Ayurvedic medications (Joy *et al.*, 1998). Based on World Health Organization (WHO) reports, more than 80% of the World population relies on traditional medicine for their primary healthcare needs (Duraipandiyan *et al.*, 2006).

Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoides, alkaloids and flavonoides which have been found *in vitro* to have antimicrobial properties (Cowan, 1999). Phenolic compounds represent a large group of molecules are widely distributed in plant kingdom and main purpose is found to be defensive. Phenolic compounds are characteristic of plants and as a group they are usually found as esters or glycosides rather than as free compounds. Phenolic compounds include signaling molecules, pigments and flavors that can attract or repel, as well as compounds that can protect the plant against insects, fungi, bacteria, and viruses. In the presented work eight plants *i.e*., *Glycyrrhiza glabra* (Papilionaceae), *Tinospora cordifolia* (Menispermaceae), *Tribulus terrestris* (Zygophyllaceae), *Thevetia peruviana* (Apocynaceae), *Adhatoda vasica* (Acanthaceae), *Vitex negundo* (Verbenaceae), *Apium graveolens* (Apiaceae), and *Annona squamosa* (Annonaceae) are selected for total phenolic contents and its antibacterial activity.

MATERIALS AND METHODS

Test organisms: Four bacterial strains i.e., two gram positive; *Bacillus cereus*, *Staphylococcus aureus*, and two gram negative; *Salmonella typhy B* (*STB*), *Shigella dysenteriae* were selected for antimicrobial activity.

Activation of culture and inoculum preparation: Active cultures were prepared by transferring a loop full of cells from the stock culture to autoclaved nutrient broth. The flasks were incubated on shaking condition at 37°C for 24 h. Next day activated cultures were used for the study. The inoculum (0.1 ml) was prepared in sterile distilled

Table 1. Total phenols of plant extracts.

No.	Plant name	mg CGA equivalent/ gm dry weight	± Standard deviation
1	*Glycyrrhiza glabra*	2.351079	0.071418
2	*Tinospora cordifolia*	1.096583	0.18809
3	*Ttribulua terrestris*	1.195504	0.142836
4	*Thevetia peruviana*	2.13705	0.054447
5	*Aadhatoda vasica*	1.621763	0.091924
6	*Vitex negundo*	1.28723	0.196576
7	*Apium graveolens*	1.324101	0.130815
8	*Annona squamosa*	1.905036	0.060104

water (0.9 ml) and 10^{-3} dilution was utilized for the crude method. For the agar ditch diffusion method, 0.1 ml of broth was mixed with 20 ml of autoclaved Mueller-Hinton agar medium.

Collection of plants: Eight plants with their selected parts *i.e.*; *Glycyrrhiza glabra* (wood), *Tinospora cordifolia* (tender), *Tribulus terrestris* (fruit), *Thevetia peruviana* (leaf), *Adhatoda vasica* (leaf), *Vitex negundo* (leaf), *Apium graveolens* (seed), *Annona squamosa* (seed) were collected from the Junagadh area, Gujarat. The application of any plant as a drug depends on the accumulation of active ingredients in a particular part. Based on literature survey (Khare, 2007) from these plants, the different parts with medicinal value (as mentioned above with their name) were separated and washed with tap water. They were surface sterilized with 0.1% $HgCl_2$ followed by three washes of distilled water and kept for air drying.

Preparation of plant extract: The air dried parts cut into small pieces and homogenized in a mechanical mortar to fine powder. These powders were used for preparation of solvent extracts. Three grams of dry powder weighed and mixed in 30 ml methanol and petroleum ether respectively and kept for 3 days at 37°C on shaking condition. The plants extracts were centrifuged at 5000 rpm and residues washed twice with respective solvents. The supernatants were kept for evaporation in dark condition. Dried evaporated extracts were dissolved in Dimethyl sulfoxide (DMSO) and final volume 5 ml was prepared (60 mg/0.1 ml). All extract were stored at 4°C and used for further studies.

Antibacterial activity: These eight plants were first screened by crude extract method for its antimicrobial property against the selected bacterial strains. Later on their antibacterial activity was studied by agar ditch diffusion method using solvent extracts. In this method 0.1 ml of bacterial culture was inoculated in 20 ml of autoclaved Mueller-Hinton agar. This is mixed properly, poured in sterile petri plate and allowed for solidification. After solidification a ditch (6 mm) was made in the centre of plate with the help of a sterile cup borer. Hundred µl test extract was introduced into the ditch and the plates were incubated overnight at 37°C 24 h. Next day results were recorded by measuring the zone of inhibition.

Extraction of phenolic compound from the plants: Two grams of dry powder was taken and to this 10 ml of 80% ethanol was added. The mixer was kept in dark for 24 h in shaking condition. Next day the extracts were centrifuged at 3000 rpm for 15 min. The supernatant was collected in petri plate and allowed for evaporation. The remaining dry residue was dissolved in sterile distilled water and final volume (5 ml) was prepared.

Assay of total phenols: The concentration of total phenols was determined with Folin-Ciocalteu reagent following the colorimetric method (Folin and Ciocalteu, 1927). Measurements were carried out in triplicate. A calibration curve of chlorogenic acid in a range of 5-50

Table 2. Antibacterial activity of methanol extracts of *Glycyrrhiza glabra*, *Tinospora cordifolia*, *Tribulus terrestris*, *Thevetia peruviana*, *Adhatoda vasica*, *Vitex negundo*, *Apium graveolens*, *Annona squamosa* against *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella typhy B* (*STB*), and *Shigella dysenteriae.*

		Zone of inhibition (mm)			
No.	**Plant name**	***Bacillus cereus***	***Staphylococcus aureus***	***Salmonella typhy B (STB)***	***Shigella dysenteriae***
1	*Glycyrrhiza glabra*	18 + 1.0	18 + 1.7	23 + 1.0	13 + 2.5
2	*Tinospora cordifolia*	13 ± 2.0	-	14 ± 1.2	16 ± 0.5
3	*Ttribulua terrestris*	14 ± 2.0	-	17 ± 2.6	11 ± 0.5
4	*Thevetia peruviana*	17 ± 1.5	13 ± 1.0	13 ± 1.0	17 ± 2.6
5	*Aadhatoda vasica*	15 ± 1.0	-	14 ± 0.5	21 ± 0.1
6	*Vitex negundo*	13 + 0.5	16 + 0.5	23 + 3.0	15 + 1.5
7	*Apium graveolens*	-	-	-	11 ± 1.1
8	*Annona squamosa*	-	-	13.3 ± 2.0	13 ± 0

Table 3. Antibacterial activity of petroleum ether extracts of *Glycyrrhiza glabra*, *Tinospora cordifolia*, *Tribulus terrestris*, *Thevetia peruviana*, *Adhatoda vasica*, *Vitex negundo*, *Apium graveolens*, *Annona squamosa* against *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella typhy B* (*STB*), and *Shigella dysenteriae.*

		Zone of inhibition (mm)			
No.	**Plant name**	***Bacillus cereus***	***Staphylococcus aureus***	***Salmonella typhy B (STB)***	***Shigella dysenteriae***
1	*Glycyrrhiza glabra*	15 ± 1.5	21 ± 1.5	14 ± 1.5	12 ± 1.0
2	*Tinospora cordifolia*	10 ± 0.5	15 ± 1.0	14 ± 0.5	10 ± 0.5
3	*Ttribulua terrestris*	-	10 ± 0.5	12 ± 0.5	12 ± 0
4	*Thevetia peruviana*	-	13.6 + 1.5	11 + 1.0	17.3 + 3.5
5	*Aadhatoda vasica*	16 ± 0.5	-	16 ± 1.5	-
6	*Vitex negundo*	15 ± 1.0	12 ± 1.0	15 ± 0.5	12 ± 1.5
7	*Apium gr aveolens*	-	18 ± 1.0	21 ± 1.1	-
8	*Annona squamosa*	-	15.3 + 2.8	15 + 2.4	15.6 + 1.6

µg/ml was prepared (Figure 1). The levels of total phenols were expressed as milligrams of chlorogenic acid equivalents per gram of dry weight (mg CGA g^{-1} DW).

RESULTS AND DISCUSSION

The use of plant extracts and phytochemicals, with established antimicrobial properties, could be of great significance in preventive and/or therapeutic approaches. In the present study, the eight plants were initially screened by crude extract method against two gram positive and gram negative bacteria for its antimicrobial property. Further their precise antimicrobial activity was tested by agar well diffusion method using different solvent extracts. This method showed significant reduction in bacterial growth in terms of zone of inhibition around the ditch. The most important exhibited antimicrobial compounds of plants are alkoloids, flavonoids, tannins and phenolic compounds (Ates and Erdogrul, 2003; Duraipandiyan *et al*., 2006). Phenolic compounds represent a large group of molecules with a variety of functions in plant growth, development, and defense (Duraipandiyan *et al*., 2006). In this study the total phenolic content from the selected medicinal plants was estimated. The probable role of total phenols in antibacterial activity was analyzed. Among the all selected plants *G. glabra* showed the maximum amount of total phenols followed by *T. peruviana and A. squamosa* (Table 1). The antibacterial activity of methanol and petroleum ether extract of these plants is presented in Table 2 and 3.

It was observed that the selected eight plants possessed relatively antibacterial activity against gram positive and gram negative bacteria. The methanol extract of *V. negundo* and *G. glabra* showed maximum zone of inhibition against *Salmonella typhy B* (*STB*) while petroleum ether extract of *A. graveolens* showed maximum zone of inhibition against (*STB*). However methanol extract of all plants except *A. graveolens* showed inhibitory activity against *STB*.

Staphylococcus aureus was highly inhibited by petroleum ether extract of *G. glabra* and *A. graveolens* followed by *A. squamosa*, *T. cordifolia*, *V. negundo* and *T. terrestris*. The presence of antimicrobial substance in the higher plants is well established. In *G. glabra* phenolic alcoholic class of compounds (flavonoids) are present which are active against *S. aureus* (Fukai *et al.*, 2002). The present study showed maximum amount of phenolic content in *G. glabra* amongst the studied plants which may lead to highest antibacterial activity. Methanol extracts of *G. glabra* exhibited potent antimicrobial activity against cariogenic bacterium *Streptococcus mutans* (Hwang *et al.*, 2004). Antimicrobial activity of *G. glabra* against gram negative bacteria was reported by Shirazi *et al*. (2007). Previous study revealed that about 100 phenolic compounds have been identified from the *Glycyrrhiza* species, many of which are isoprenoid substituted phenols (flavonoids). Some of these flavonoids have shown inhibitory activities against bacterial growth (Hsieh and Wu, 2002; Fukai *et al.*, 2004; Jain *et al.*, 2006). Methanol extract of *T. cordifolia*, *T. terrestris*, *A. vasica*, *A. graveolens* and *A. squamosa* was not showing inhibition against *S. aureus.* Earlier it was demonstrated that ethanolic extracts of the fruit and leaf of Indian *T. terrestris* were active against *Escherichia coli* and *S. aureus* (Williamson, 2002). However different parts of Turkish and Iranian *T. terrestris* have been reported to have antibacterial activity (Abbasoglu and Tosun, 1994; Kianbakht and Jahaniani, 2003).

In comparison to petroleum ether the methanol extract of all eight plants showed inhibitory activity against

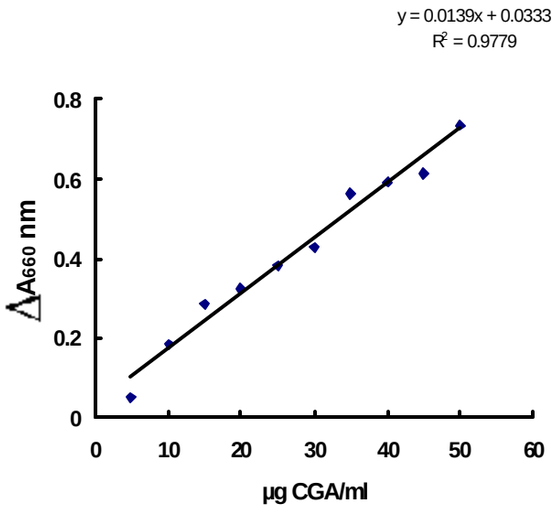

Fig. 1. *A calibration curve of chlorogenic acid.*

Shigella dysenteriae. *S. dysenteriae* was highly inhibited by methanol extract of *A. vasica* followed by *T. peruviana, T. cordifolia, V. negundo,* G. *glabra, T. terrestris,* and *A. graveolens.* Both extracts of *G. glabra, T. cordifolia, V. negundo* and *A. vasica* showed inhibition against *B. cereus*. However the zone of inhibition remained higher by methanol extracts of *G. glabra* and *T. cordifolia* plants. Methanol extract of *T. peruviana* showed maximum zone of inhibition against *Bacillus cereus* and *Shigella dysenteriae*, while its inhibitory activity was similar to *S. aureus* and *S. typhy B* (Table 2). The results showed that the methanol extract of all test plants has more inhibitory effect. This tends to express that the active ingredients of the plant parts may be better extracted with methanol than other solvents (Babu *et al.*, 2007).

The present study revealed that all tested plant extracts were effective against gram negative bacterial species. Instead of both solvent extracts methanol extracts exhibited a higher degree of antimicrobial activity compared to petroleum ether extract. Roberts *et al*. (1981) demonstrated that the antibacterial activity of methanol extract is due to the presence of phenolic and acidic compounds. Among the studied plants *G. glabra* and *V. negundo* with their both extracts remained highly inhibitory against the four selected bacteria. Higher amount of phenolic content of *G. glabra*, *T. peruviana* and *A. squamosa* lead to their antimicrobial property. The antimicrobial activity of phenols is well established (Paupponen *et al.*, 2001 and Pansuria *et al.*, 2006). The antimicrobial activities of naturally occurring phenolics from olives, tea and wine have been widely studied (Ruiz-Barba *et al.*, 1990; Vivas *et al.*, 1997 and Chou *et al.*, 1999). Based on the results, it is concluded that methanolic extracts have great potential as antimicrobial compounds against microorganisms and they can be used in the treatment of infectious diseases caused by the selected microorganisms.

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