Screening of plants for antibacterial activity against *Shigella* species

Satish S.1,2,*, Raghavendra M P.1,3, Raveesha K A 1

1 Herbal Drug Technology Laboratory, University of Mysore, Mysore, Karnataka, India
2 Microbiology Laboratory, University of Mysore, Mysore, Karnataka, India
3 Dept. of Microbiology (PG), Maharani’s Science College for Women Mysore, Karnataka, India

Submitted: 13 Aug. 2009; Revised: 30 Dec. 2009; Accepted: 7 Jan. 2010

**Abstract**

Aqueous extracts of leaves of 48 plants belonging to 33 different families of the plant kingdom were subjected to *in vitro* antibacterial activity assay against human pathogen *Shigella boydii*, *Shigella flexneri* and *Shigella sonnei* employing cup diffusion method. The selection of plants was based on traditional knowledge and random selection. The results revealed that aqueous extract of 10 plants (21%) exhibited antibacterial activity. Different solvent extracts viz., petroleum ether, benzene, chloroform, methanol and ethanol extracts of all the plants were also subjected to antibacterial activity. Among the different solvent extracts tested methanol extract of 13 plants (27%) revealed antibacterial activity and the spectrum of activity varied among the test pathogens. The minimal inhibitory concentration of aqueous extracts varied for the *Shigella* species tested. The present study demonstrates a collection of candidate plants with anti-*Shigella* activity, which could be further exploited for isolation and characterization of the active principle and management of diseases caused by species of *Shigella*.

**Keywords**: Antibacterial activity, *Shigella* species, plant extracts.

**INTRODUCTION**

Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer *et al*., 1999). It has been estimated that 14-28% of higher plant species are used medicinally and that 74% of pharmacologically active plant derived components were discovered world wide after following up on ethnomedicinal use of the plants. In India, about 3000 plants are used in the form of ethno- or folk-medicines based on oral information from generation to generation. About 1500 plants are systematically used in indigenous system of medicine, like Ayurveda, Unani and Siddha. However, the ethnopharmacologists, botanists, microbiologists and natural-product chemists world over today are constantly in search of medicinal efficacy of plants and their phytochemicals, since the reported data so far available on plants are less despite their huge antibacterial potential. The other reasons also includes search for novel phytochemicals which could be developed for the treatment of infectious diseases especially in light of the emergence of drug-resistant microorganisms and the need to produce more effective antimicrobial agents (Tanaka *et al*., 2006).

The search is successful only with thorough biological evaluation of plant extracts which is vital to ensure their efficacy and safety and also will serve as a preliminary tool for the development of pharmaceuticals (Iwu, 1999). It is obvious that folk medicines are the original source to point out the plants as the source of medicine in the primary level (Prakash, 1998).

The drugs which are already in use to treat infectious diseases is of concern because, drug safety remains an enormous global issue. It was estimated that 2.22 million hospitalized patients had serious Adverse Drug Reactions (ADR) and 106,000 died in a single year in the USA. This implied that ADRs were a major cause of death ranking between 4th and 6th in the list of most common causes of death. In UK also ADR accounted for around 6.5% of hospital admission and 4% of the hospital bed capacity (Lazarou, 1998). This along with multiple therapeutic options available for most common diseases made selection of the optimal medication for the individual patient an open question (Islam, 2008). Herbal and natural products have been

*Corresponding author:
Satish S., Ph.D.
Microbiology Laboratory
Department of Studies in Microbiology
University of Mysore, Manasagangotri,
Mysore- 570 006, Karnataka, India.
Email: satish.micro@gmail.com
used in folk medicine for centuries throughout the world, but there are relatively lower incidences of adverse reactions to plant preparations compared to modern conventional pharmaceuticals. This coupled with their reduced cost, encourages both the patients and national health care institutions to consider plant medicines as alternatives to synthetic drugs (Nair et al., 2005).

In view of all these, thorough screening of plants for antibacterial activity against three species of Shigella viz., Sh. boydii, Sh. flexneri and Sh. sonnei was carried in our lab. The screening was initiated with aqueous extract, as the selection of crude plant extracts for screening programs has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products (Kusumoto et al., 1995).

The pathogens selected for this investigation are Shigella species that cause bacillary dysentery. For this pathogen antibiotic resistance to sulphonamides, streptomycin, tetracyclines, ampicillin and chloramphenicol and even strains resistant to trimethoprim are reported (Gross et al., 1984). Several multi drug resistant Shigella have remained a cause of concern in endemic regions (Thirunarayanan et al., 1993). Nalidixic acid resistance has increased for Shigella sonnei in some of the Indian isolates. This is of concern because it is commonly used for the empirical treatment of patients suspected to have shigellosis (Jesudason, 2002). Kapil (2005) has suggested that the man made antibiotic pressure leads to the emergence and spread of resistant genes among bacteria. Despite the availability of a large arsenal of antibiotics, the ability of bacteria to become resistant to antibacterial agents is of concern. In view of this, the aim of this research work is to investigate antimicrobial potential of the extracts of some plants as herbal medicine on the growth of Shigella species.

**MATERIALS AND METHODS**

**Collection of plant material**

Fresh disease free leaves of forty four plant species belonging to 33 different families of the plant kingdom (Table 1 [Supplementary data]) were collected from Mysore, Karnataka, India. The leaves were washed thoroughly several times with running tap water and once with sterile distilled water. The leaf material was then air-dried on a sterile blotter under shade. A voucher specimen of all the plants has been deposited in the herbarium of Department of Studies in Botany, University of Mysore, Mysore.

**Preparation of extracts**

**Aqueous extract**

Leaf samples (50 g) of the plants were thoroughly washed, blot dried and macerated with 100 ml sterile distilled water in a waring blender (Waring international, New Hartford, CT, USA) for 10 min. The macerate was first filtered through double layered muslin cloth and then centrifuged at 4000 g for 30 min. The supernatant was filtered through Whatmann No. 1 filter paper and heat sterilised at 120ºC for 30 min. These extracts which served as a mother extract were allowed to cool to room temperature and their pH was determined just before subjecting it for antibacterial activity assay. The extracts were preserved aseptically in sterile brown bottles at 5ºC until further use.

**Solvent extracts**

Thoroughly washed mature leaves were shade dried and then powdered with the help of waring blender. Twenty-five grams of the powder was filled in the thimble and extracted successively with petroleum ether (30-60ºC), benzene (80.1ºC), chloroform (61.2ºC), methanol (64.6ºC) and ethanol (78.5ºC) using a Soxhlet extractor for 48 hrs. All the extracts were concentrated using rotary flash evaporator and preserved at 5ºC in airight bottle until further use. All the extracts were subjected to antibacterial activity assay. For each treatment 16 replicates were maintained.

**Bacterial cultures**

Clinical isolates of Shigella boydii, Shigella flexneri and Shigella sonnei were obtained from the Department of Microbiology, Government Medical College, Mysore, Karnataka, India. All the test strains were maintained on nutrient agar slopes (Hi-Media) and were subcultured once every two-week. These bacteria served as test pathogens for antibacterial activity assay.

**Antibacterial activity assay**

Antibacterial activity of the different extracts was determined by cup diffusion method on nutrient agar medium (Anonymous, 1996). Cups are made in nutrient agar plate using cork borer (5 mm) and inoculums containing 10⁶ CFU/mL of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. Then 2-50 µL concentration of the aqueous extract and different solvent extracts were placed in the cups made in inoculated plates, the treatments also included 50 µL of sterilized distilled water and respective solvents alone, which served as control. All the plates were incubated for 24 hrs at 37ºC and zone of inhibition if any around the wells after incubation was measured in mm (millimeter). For each treatment 12 replicates were maintained.

Six widely used antibiotics - ampicillin, bacitracin, ciprofloxacin, gentamicin, penicillin G and
against Five plants recorded significant antibacterial activity in the plants. The concentration of aqueous extract was observed in all Significant increase in activity with increase in inhibitory activity was observed only above 8 µL extracts at different concentrations tested. The extracts of eight plants were acidic, one was basic and 10 plants which recorded inhibitory activity aqueous extract of only five plants were found to have inhibitory activity against S. sonnei, whereas other plants did not record activity. At 50 µL concentration aqueous extract of Achras zapota was highly significant followed by other plants (Table 2).

**Determination of Minimal Inhibitory Concentration (MIC)**

MIC was determined by broth dilution methods. 0.1 ml of standardized suspension of test bacteria (10^6 CFU/ml) were inoculated to series of culture tubes (microdilution assays) (Ferreira et al., 2003) with same volume of medium containing different concentrations of the aqueous extract (200-1000 µg/ml). The lowest concentration of sample at which the subculture from test dilution yielded no viable organisms was recorded as minimum bactericidal concentration (Nazaruk and Jakoniuk, 2005). Each assay was performed in quadruplet. One tube was left without aqueous extract to serve as positive control and other without aqueous extract and inoculum to serve as negative control. The cultures were incubated at a temperature optimal for growth of the test organism and a period of time sufficient for growth for at least 10-15 generations (usually 24 hrs for bacteria at 37 °C). The tubes were inspected visually to determine the growth of organisms by the presence of turbidity and the tubes in which aqueous extract is present in minimum concentration sufficient to inhibit the microbial growth which remains clear was noted as MIC of the extract.

The data was subjected to statistical analysis using SPSS.

**RESULTS**

Aqueous extract show varied degree of pH. Among 48 plants, 26 plants yielded aqueous extract with acidic pH, 12 with basic pH and 10 with neutral pH. Among the 10 plants which recorded inhibitory activity aqueous extract of eight plants were acidic, one was basic and one was with neutral pH (Table 1).

Among the 48 plants tested, 10 plants (21%) recorded inhibitory activity against one or more pathogens used (Table 1). Antibacterial activity varied for the plant extracts at different concentrations tested. The inhibitory activity was observed only above 8 µL concentration and highly significant activity was observed at 50 µL concentration. Statistically significant increase in activity with increase in concentration of aqueous extract was observed in all the plants.

**Antibacterial activity of aqueous extract**

Five plants recorded significant antibacterial activity against S. boydii, in which aqueous extract of Achras zapota recorded highly significant antibacterial activity followed by Acacia nilotica, Lawsonia inermis, Viscum orientale and Oxalis corniculata at 50 µL concentration. At the same concentration, aqueous extract of Oxalis corniculata recorded highly significant antibacterial activity against S. flexneri followed by Emblica officinalis, Punica granatum, Achras zapota, Acacia nilotica, Syzygium cumini, Viscum orientale and Lawsonia inermis (Table 2 [Supplementary data]).

Only four plants viz., Achras zapota, Lawsonia inermis, Acacia nilotica and Viscum orientale recorded antibacterial activity against S. sonnei, whereas other plants did not record activity. At 50 µL concentration aqueous extract of Achras zapota was highly significant followed by other plants (Table 2).

**Minimal Inhibitory Concentration (MIC)**

Aqueous extract of all the 10 plants showed varied MIC against Shigella species tested. MIC varied between 400 to 650 µg/ml among the aqueous extracts of plants tested against S. boydii. The least 400 µg/ml was observed for Acacia nilotica and 650 µg/ml of Syzygium cumini was the maximum. The range of MIC was similar for S. Flexneri. The minimum 400 µg/ml was observed for Acacia nilotica, Emblica officinalis, Manilkara zapota, Oxalis corniculata and Punica granatum and the maximum 650 µg/ml was for Lawsonia inermis.

Among the several plants tested aqueous extract of only five plants were found to have inhibitory activity against S. sonnei. The MIC varied between 500 to 700 µg/ml for these plants. The minimum was Acacia nilotica with 500 µg/ml and maximum was Syzygium cumini (Table 3 [Supplementary data]).

**Antibacterial activity of solvent extracts**

Among different solvent extracts tested, only methanol extract of 13 plants (30%) recorded significant antibacterial activity followed by ethanol extract, where as no activity was observed in other solvent extracts. Methanol extract of Tamarindus indica recorded highly significant activity against S. boydii among 13 plants followed by Lawsonia inermis, Acacia nilotica, Anacardium occidentale and Oxalis corniculata, where as ethanol extract of Lawsonia inermis recorded highly significant activity compared to ethanol extracts of other plants (Table 4 [Supplementary data]).

Methanol and ethanol extracts of Tamarindus indica recorded highly significant antibacterial activity against S. flexneri followed by Lawsonia inermis, Acacia nilotica, Oxalis corniculata and Achras zapota, where as only methanol extract of Emblica officinalis, Anacardium occidentale, Samanea saman was found inhibitory (Table 4). Methanol extract of Tamarindus indica recorded highly significant antibacterial activity followed by Acacia nilotica, Oxalis corniculata,
Achras zapota, Lawsonia inermis, Emblica officinalis, Samanea saman and Anacardium occidentale, where as ethanol extract of Emblica officinalis, Anacardium occidentale, Samanea saman did not record significant activity, among the activity found Acacia nilotica recorded highly significant activity (Table 4).

**Antibacterial activity of antibiotics**

Ciprofloxacin and gentamicin recorded inhibitory activity against all the species of *Shigella*, where as penicillin G, streptomycin and bacitracin did not show activity against all the three test pathogens. Ampicillin was found inhibitory only against *S. sonnei* and no activity was observed against other pathogens (Table 5 [Supplementary data]).

The comparative evaluation of aqueous and methanol extract with antibiotics revealed encouraging result. *Shigella* species tested were found resistant to three antibiotics viz., bacitracin, penicillin G and streptomycin. The same was true with ampicillin (except against *S. Sonnei*). Among the different antibiotics tested ciprofloxacin recorded highly significant antibacterial activity against all the test pathogens even compared to aqueous and methanol extract of all the plants, gentamicin recorded significant activity next to ciprofloxacin against *S. sonnei* and *S. boydii* but *S. flexneri* for which aqueous extract of *Acacia nilotica, Emblica officinalis, Manilkara zapota, Oxalis corniculata, Punica granatum, Syzygium cumini* and *Viscum orientale* and methanol extract of *Acacia nilotica, Lawsonia inermis, Oxalis corniculata* and *Tamarindus indica* were found highly significant.

**DISCUSSION**

The beneficial medicinal effects of plant materials typically result from the secondary products present in the plant (although it is usually not attributed to a single compound but a combination of the metabolites). The medicinal actions of plants are unique to a particular plant species or group, consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct (Parekh et al., 2005). These secondary products on a global basis are responsible for the discovery of 130 drugs, currently in use, all single chemical entities extracted from higher plants, or modified further synthetically,. Some of them are now being made synthetically for economic reasons (Newman et al., 2000). Large scale evaluation of the local flora exploited in traditional medicine for various biological activities is a necessary first step in the isolation and characterization of active principle and further leading to drug development. Such screening revealed antibacterial activity of plant extracts against wide range of bacteria including antibiotic resistant bacteria and *Shigella* sp. (Nascimento et al., 2000; Alam et al., 2006; Rahman et al., 2008; Alam et al., 2009). Hence in the present investigation with the goal of identifying a candidate plant for anti-shigella compounds, 48 plants belonging to 33 different families of the plant kingdom were screened against *Shigella* species. All the plant material was collected from Mysore district. This is the first report of screening a wide range of plants from Mysore district, Karnataka State, India for antibacterial activity against *Shigella* species.

The screening of plants usually involves several approaches; ethnobotanical approach is one of the common methods that is employed in choosing the plant for pharmacological study. In a world gifted with biodiversity, it is impossible to screen each of the plant species for biological activity (Cox and Balick, 1994). Hence, it was suggested that the investigators can gather vegetation randomly in an area supporting rich biological diversity, even though random searches yield relatively few new drug possibilities. Considering these, in the present investigation 48 plants were selected based on both traditional knowledge and random selection from the local flora in order to screen for their antibacterial potency against *Shigella* sp. Among the plant tested thirteen plants were found to possess antibacterial activity against *Shigella* species. The screening is involved angiosperm parasites *Macrosolen parasiticus*, the inhibitory activity is reported for the first time from the present investigation.

Among the several plants screened, aqueous extract of most of the plants show acidic pH and even among the 13 plants which recorded antibacterial activity eight plants were acidic, the present study is the first to describe the pH of aqueous extract of several plants which indirectly indicates the possible presence of more metabolites which are acidic in nature. The important outcome of the study is that all the strains were found resistant to three important antibiotics such as bacitracin, penicillin G and streptomycin and were found to be susceptible to the plant extracts indicating that plant extracts have different mode of action compared to already available antibiotics.

*Tamarindua indica* which yielded highly acidic aqueous extract did not record antibacterial activity against *S. boydii, S. sonnei*, and activity against *S. flexneri* was also not highly significant. But it is quite interesting to observe that methanol and ethanol extract of same plant recorded highest activity against all the species compared to all the 13 plants. This observation is also true with *Oxalis corniculata* against *S. Sonnei* where, aqueous extract did not record inhibitory activity when compared to methanol extract which recorded highly significant activity. The other observation is that the antibacterial activity is pronounced in methanol extract of *Acacia nilotica, Lawsonia inermis, Samanea saman, Emblica officinalis*...
compared to aqueous extract, inversely less activity was observed in solvent extracts of 
_Achras zapota_ compared to aqueous extract against all the _Shigella_ species, where as no difference in activity was observed in extracts of _Prosopis juliflora_, _Punica granatum_ and _Syzygium cumini_. In case of _Viscum orientale_ aqueous extract recorded significant antibacterial activity against all the pathogens compared to solvent extracts. These observations are of high importance as the extraction procedure of the plant material need to be planned, since the plant metabolites of non polar nature are easily extracted by organic solvents compared to water. The possible influence of organic solvent and extraction procedure on stability or nature of the plant metabolites/active principle is also a subject of debate.

The present study demonstrates the antibacterial activity of several plants which could be exploited in human disease management in general and diseases caused by _Shigella_ in particular. The study is also successful in identifying plants which could be subjected for further isolation and characterization of bioactive molecules responsible for anti-shigella activity. Further investigations are in progress in our laboratory.

**Acknowledgement**

The authors are grateful to the Department of Science and Technology (DST), New Delhi for financial assistance (Grant Number: SR/FT/LS-023/2007). The authors are also thankful to Prof. Shivismurthy G.R., Professor (Retd.), Taxonomist, Department of Studies in Botany, University of Mysore, Mysore for helping in identification of plant species.

**References**


