Shigella spp., the test pathogen; its procurement and identification:

Different workers have selected or tested different test pathogens without ascribing any reason of selection during antibacterial investigation (Table 1 & 2). In the present study Shigella spp., has been chosen as test pathogen as it is very notorious and multipronged pathogen which causes bacillary dysentery very frequently in human beings. Hence, a safe means of control has to be found. The procurement of the pathogen was made directly from the patients by collecting samples from them and then the samples were cultured in the laboratory. It ensured the authenticity of the pathogen and further the test pathogen was identified by various morphological, cultural and biochemical studies. Identification of the test pathogen was given due importance.

Screening of higher plants for antibacterial activity:

A perusal of literature shows that a large number of higher plants belonging to Angiosperms and Gymnosperms have been screened for their antibacterial activity (Table 1 and 2). Mostly the aqueous extracts or expressed juices of plants have been used to evaluate their bacteritoxicity (Kaysei and Kolodziej, 1997; Bae et al., 1998; Abo et al., 1999; Kokosoka and Rada, 2000; Setzer et al., 2001;
Bardhanand Chatterjee, 2002; Gibbons et al., 2002; Newton et al., 2002; Adeniyi and Anyiam, 2004; Garud et al., 2004; Lee et al., 2004 and Germano et al., 2005).

Aqueous extract or expressed juices may lose their efficacy due to degradation of active constituents by continued enzymatic activity. Enzymes may come along with extract and juices. In the present investigation 50% ethanol has been used which ensures extraction of maximum compounds as well as facilitates further purification of active fractions (Dhar et al., 1973; Kumar et al., 1995; Xu and Lee, 2001; Shanmuga Priya et al., 2002; Turker and Camper, 2002 and Santhamarai et al., 2003). A number of workers have screened plants which are used in folk medicines or as herbal medicines only (Bae et al., 1998; Chakraborty and Chattopadhyay, 1998; Alasbahi et al., 1999; Audu et al., 2000; Feresin et al., 2000; McGraw et al., 2000; Mishal and Somani, 2000; Ahmad et al., 2000-2001; Srinivasan et al., 2001; Neto et al., 2002; Kokoska et al., 2002; Mangathayaru et al., 2004; Rani and Khullar, 2004; Billo et al., 2005 and Zaidi and Crow, 2005). Some workers have screened plants of specific families only (Binutu, 1998; Habsah et al., 2000; Jantova et al., 2000; Rosado-Vallado et al., 2000; Ali et al, 2001; Karuppuswamy et al., 2001 and Jayasinghe et al., 2002). Rao et al., 1946; Datta et al., 1948; Nutan et al., 1998; Ragasa et al., 1999; Binutu and
A number of workers have screened the stem bark...
for bactericidal activity (Abo et al., 1999; Elsohly et al., 1999; Ajali, 2000; Bhandari et al., 2000; Jumana et al., 2000; Mandal et al., 2000; Omar et al., 2000; Rabe and van Staden, 2000; Conrad et al., 2001; de M. Schlemper et al., 2001; Ebi and Kamalu, 2001; Pillay et al., 2001; Rahman and Gray, 2002; Zgoda et al., 2001; Abdelrahim et al., 2002; Fokialakis et al., 2002; Nandha Kumar et al., 2002; Jeyachandran et al., 2003; Parcha et al., 2003; Dinda et al., 2004; Jain et al., 2004 and Mahadevan et al., 2004.

Flaimini et al., 1999; Deena and Thoppil, 2000; Aligiannis et al., 2001; Calcuttawalla et al., 2002; Demo et al., 2002; de Abreu Gonzaga et al., 2003; Laouer et al., 2003; Aligiannis et al., 2003; Mirjana and Nada, 2004 and Al-Burumanani et al., 2005, have screened only aerial parts of the plant. The workers had selected a particular plant part or plants of notified medicinal importance only, for antibacterial assay. In the present study all the available plants (and their parts) of the locality, irrespective of particular family or medicinal importance had been assayed for antibacterial activity and it was found that leaves of *Adhatoda vasica* had strongest bactericidal activity than its root, stem, flower and fruit.

The antibacterial plant *Adhatoda vasica*, chosen for detailed investigation, is distributed throughout the tropical part of India. This plant is important from medicinal point of view, but no report has been made as yet, for its antibacterial activity against *Shigella spp.*
Effect of various physical factors on the activity of active plant parts:

The effect of various physical factors on antibacterial activity of active fraction has received little attention. The effect of storage time on the antibacterial activity has been studied and it was found that the activity persisted 15 days only in the leaves of Adhatoda vasica, though experimentation was done upto 30 days. The loss in the activity may be due to some general reactions like oxidation, isomerisation, polymerisation, hydrolysis of esters or interaction of functional groups etc.. The antimicrobial activity of the active fraction from the leaves of Adenocalymma allicea has been found stable for 21 days only, after which it declined (Chaturvedi, 1979). Saxena (1980) found that the active fraction of Putranjiva roxburghii lose its antimicrobial activity gradually after 33 days of storage. Active fraction of Achyranthes aspera has been found to be comparatively less stable (Yadav, H.L., 2005).

The antimicrobial principles, isolated from seed coats of Sorghum vulgare, Eleucine coracana and Lycopersicum esculentum were found thermostable upto 100°C (Balasubramanian and Rangaswamy, 1967). Chaturvedi (1979) observed that the antimicrobial volatile oil of Adenocalymma allicea retained activity upto 50°C. In the present investigation the antibacterial activity in the leaves of Adhatoda vasica
was found thermostable up to $40^\circ C$ and then it gradually lost its activity at $60^\circ C$. On autoclaving at $121^\circ C$ the leaves lost the activity at all.

Several workers have isolated different compounds from various plants and after that their antimicrobial activity was tested \textit{in vitro} (Yadava, 1989; Sadyojatha and Vaidya, 1996; Verma \textit{et al.}, 1997; Alvarea \textit{et al.}, 1999; Cantrell \textit{et al.}, 1999; Chae \textit{et al.}, 1999; Chen \textit{et al.}, 1999; Elsohly \textit{et al.}, 1999; Binutu and Cordell, 2000; Kuroyanagi \textit{et al.}, 2000; Chowdhury \textit{et al.}, 2001; Galal \textit{et al.}, 2001; Kawazoe \textit{et al.}, 2001; Fokialakis \textit{et al.}, 2002; Anjum \textit{et al.}, 2002; McGaw \textit{et al.}, 2002; Zhang \textit{et al.}, 2002; Cattiglia \textit{et al.}, 2004; Graham \textit{et al.}, 2004 and Kanokmedhakul \textit{et al.}, 2004). On the other hand some other workers first tested the antimicrobial activity of plants and if they were found antimicrobial then fractionation was done and each fraction was assayed against the test pathogen to find out the active fraction (Chakraborty & Patil, 1997; Nutan \textit{et al.}, 1998; Ragasa \textit{et al.}, 1999; Ajali, 2000; Audu \textit{et al.}, 2000; Deng \textit{et al.}, 2000; Jumana \textit{et al.}, 2000; Lall and Meyer, 2000; Lindsay \textit{et al.}, 2000; Madhumathi \textit{et al.}, 2000; Mosaddik \textit{et al.}, 2000; Pichai \textit{et al.}, 2000; Pitchai & Saraswathy, 2000; Khan \textit{et al.}, 2001; Sukul and Chaudhury, 2001; Ramesh \textit{et al.}, 2002). In the present investigation the ethanolic extract of \textit{Adhatoda vasica} was fractionated by the differential solubility method as
followed by Dixit et al., 1976 and Tripathi et al., 1978. After fractionation all the fractions were assayed against the test pathogen and only petroleum ether fraction was found to possess antibacterial activity.

Minimum inhibitory concentration of some compounds or extracts has been studied by different workers and they have found considerable variation. Kaysei and Kolodziej (1997) tested the extracts of Pelargonium spp. and isolated constituents against 3 Gram-positive and 5 Gram-negative bacteria and recorded their MICs between 200-1000 micro g/ml. Flavonoids and saponins of leaf and stem bark of Uvaria cordata inhibited Staph. epidermidis and Bacillus cereus and their MICs ranged between 0.01-3 mg/disk (Khan et al., 1998). Contrell et al., (1999) isolated the compounds from Melia volkensii seeds and evaluated the MICs of compound number 1, 2 and 4 to be 16, 4 and 16 micro g/ml. respectively against Mycobacterium tuberculosis. Elsohyl et al., (1999) isolated some compounds from Colubrina retusa and the compound number 4 (MIC-10 microg/ml.) inhibited Mycobacterium tuberculosis. Quarenghi et al., (2000) found that floral extract of Anthemis cotula inhibited Gram-positive and Gram-negative bacteria at the concentration 200 micro g/ml.. Sansores- Peraza et al., (2000) isolated an antibacterial alkaloid from the leaves of Senna racemosa which
inhibited the tested bacteria at MIC 2.5 mg./ml. and the tested fungi at
MIC 5.0 mg./ml. Several compounds of different plant parts have been
reported by different workers to show antimicrobial activities at MICs in
the range 25-127 micro g/ml. (Rahman and Gray, 2002; Austin et al.,
2003; Parcha et al., 2003; Annapurna et al., 2004; Cu et al., 2004;
Singh et al., 2004 and Billo et al., 2005). In the present study emphasis
has been given to find out the minimum inhibitory dose (MID) by the
inhibition zone technique. In this technique the concentration of
compounds decreases gradually as it diffuses out from the sensitivity
disk to the periphery. Though the disks impregnated with 4, 6, 8, 10
and 12 mg. contents of active fraction formed inhibition zones around
them, all the zones except 12 mg.-disk-zone, were gradually
invaded by the test pathogen after 72 hrs. However, zone around 12 mg.
disk persisted and remained unaffected by the test pathogen throughout
the experimentation period. So it was concluded that the 4, 6, 8, and
10 mg. disks were bacteriostatic upto 36 hrs. only, but the 12 mg.-disk
proved bactericidal during the experimentation period (72 hrs.), if one
loopful inoculum was taken to seed the 3” diameter Mac Conkey agar
plate. Here, it is required to mention that after seeding the Mac Conkey
agar plate, the number of bacteria would increase on the medium
surface and it is difficult to count their exact numbers, although Skinner
(1955) has mentioned that the efficacy of antibiotics depends upon the number of bacteria they have to act on. However to fix the exact dose requires further investigations.

**Comparison of active fraction and commercial antibiotics:**

Little attention has been paid by the workers to compare the antibacterial activity of the active fraction of plants with the activity of commercial antibiotics. Bhandari *et al.*, (2000) found that the alkaloid fraction of the stem bark of *Berberis asiatica* was more active than the alkaloid berberine against the tested bacteria. Kawazoe *et al.*, (2001) isolated some compounds from the subterranean part of *Vitex rotundifolia* and found that they inhibited methicillin-resistant *Staph. aureus* i.e. the isolated compounds were more active than the antibiotic methicillin. Shin *et al.*, (2000) have also found that the sesquiterpenoid, isolated from *Ulmus davidiana* var. *japonica*, were more active than the antibiotic methicillin and it inhibited methicillin-resistant *Staph. aureus*. Ebi and Kamalu (2001) fractionated a Nigerian herbal medicine ‘Ogwu Odenigbo’ and compared the steroidal fraction with penicillin and chloramphenicol antibiotics. He found that this fraction was 20 times more active than penicillin and 15 times more active than chloramphenicol.
Likewise several workers have investigated antibacterial activity in different plants and have compared it with standard antibiotics (Duraiswamy et al., 2002; Mukhtar et al., 2002; Annapurna et al., 2004 and Mangathayaru et al., 2004). In the present study the activity of the active fraction of Adhatoda vasica, at its MID, was compared with the activity of 6 potent broad spectrum antibiotics and it was found 2.33 times more active than Penicillin G; about two times more active than Ciprofloxacin and Kanamycin; about 1.5 times more active than Cloxacillin and Lincomycin and it was equivalently potential, rather some more active, as the antibiotic Erythromycin. The comparison was done by the prescribed antimicrobial susceptibility testing method. Simultaneously periodical observations (12 hourly) for 72 hrs. proved that the active fraction of Adhatoda vasica, at its MID, was bactericidal because the inhibition zone around it remained persistent and unaffected throughout the period of the experiment, where as the inhibition zones around commercial antibiotic sensitivity disks were invaded by the test pathogen, proving them to be bacteristatic. Over all the active fraction of Adhatoda vasica at its MID was found to be more active and competant than the commercial antibiotics.

**Bactericidal spectrum of active fraction:**

An antibacterial substance may either be specific
or broad spectrum. Tomatin, the antibiotic from the leaves of tomato plant, showed partial antibacterial spectrum (Irving et al., 1946). Nair and Bhide (1996) found that alcoholic extract of dry nuts of *Semecarpus anacardium* inhibited two Gram-positive and three Gram-negative bacteria where as the leaf extract was broad spectrum which inhibited all the tested bacteria. Verma et al., (1997) found that flavone glucoside, compound of the leaves of *Lantana camara*, showed antibacterial activity against a wide range of Gram-positive and Gram-negative bacteria. Chloroform extract of the leaves of *Murraya koenigii* inhibited *Bacillus cereus* totally and to some extent to other test bacteria (Nutan et al., 1998). Chae et al., (1999) found that the root alkaloid of *Coptis japonica* inhibited four human intestinal bacteria. The methanol extract of *Litsea glutinosa* bark was found to be broad spectrum antibacterial agent (Mandal et al., 2000). Mosaddik et al., (2000) found that the methanol extract of *Alangium salvifolium* flowers showed a wide spectrum of antibacterial activity against both Gram-positive and Gram-negative bacteria. Khan et al., (2001) found that the methanolic extracts of leaves, stem and root of *Castanopsis acuminatissima* showed broad spectrum antibacterial activity. Chloroform and methanol extracts of the leaves of *Begonia malabarica* inhibited all the tested Gram-positive and Gram-negative
bacteria (Ramesh et al., 2002). Vidya and Vidya (2002) used the mixture of essential and fixed oils of different plants and found that the mixture at low concentration inhibited *Staph. aureus* and *Salmonella spp.* only but it could not check *Pseudomonas aeruginosa, Proteus sp.* and *Klebsiella*. Aquil and Ahmad (2004) found antibacterial activity in different parts of six plants against methicillin-resistant *Staph. aureus* and all these were proved broad spectrum. In the present study the bactericidal spectrum of the active fraction of *Adhatoda vasica* was tested against 7 available human pathogenic bacteria and it was found that the active fraction more or less inhibited all the tested bacteria (six Gram-negative and one Gram-positive), proving it to be a broad spectrum antibacterial agent.

On reviewing the present investigation it has been inferred that the leaves of *Adhatoda vasica* possess strong antibacterial activity, broad antibacterial spectrum, superiority over several commercial antibiotics, can be exploited as a potent source of bactericide against human pathogens, particularly *Shigella spp.*. It could be a potent source of a safe medicine, as the compounds of plant origin are easily biodegradable and have little or no side effects on human beings. Fixation of dose and course of treatment need further investigations and pharmacological studies to enunciate the utility of this plant.