# SCREENING OF PLANT EXTRACTS USED IN TRADITIONAL ANTIDIARRHOEAL MEDICINES AGAINST PATHOGENIC *ESCHERICHIA COLI*

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**Abstract:** The petroleum ether, chloroform, methanol and aqueous extracts of *Acacia catechu* L. f. (bark), *Acacia nilotica* L. (bark), *Aegle marmelos* L. Correa. (fruit) *Azadirachta indica* L. (leaves), *Annona squamosa* L. (leaves), *Trachyspermum ammi* L. (seeds), *Holarrhena antidysenterica* L. (bark) and *Ocimum basilicum* L. (leaves) were tested against Enteropathogenic *Escherichia coli* (EPEC). These are the plants traditionally used by rural populace of semi arid regions of India for the treatment of diarrhoea. The ethnopharmacological information on the plants was collected by interviewing the traditional healers, community leaders and rural people of Gujarat State. The agar-well diffusion assay method was used to access the activities of plant extracts against the test organism. The results obtained show the strong activity of petroleum ether extract of *A. marmelos, A. indica, T. ammi* and *H. antidysenterica*; chloroform extract of *A. catechu, A. indica* and *T. ammi;* and methanol extract of *A. catechu, A. nilotica, A. marmelos* and *T. ammi* (MIC, d" 50 ì g/ml) followed by petroleum ether extract of *O. basilicum* and chloroform extract of *A. nilotica, A. marmelos* and *H. antidysenterica* (MIC, 50-100 ì g/ml). These preliminary results will be helpful in rationalizing the use of plants based traditional medicines in modern systems of health care.

**Key words:** Medicinal plants; Herbal extracts; Traditional medicines; *Escherichia coli*.

## 1. INTRODUCTION

Diarrhoea disease caused by Enteropathogenic *Escherichia coli* (EPEC) are the major reasons of morbidity and mortality among children in the developing countries (Shoba and Thomas, 2001; Dean et al., 2006). This may be attributed to the increasing resistance of pathogen to the common antibiotics (Pinner et al., 1996; Senda et al., 1996; Archibald et al., 1997; Fridkin et al., 2002; Rajani et al., 2002) and their side effects (Krolczyk et al., 2004; Bombard et al., 2005). In recent years, special attention is being given on alternative safe natural bio-remedies to cure the infectious diseases because of their less or no side effects and resistance in microbes against them (Bonjar, 2004).

Many investigations proved the treatment and deterrence of gastrointestinal diseases using the plant extracts (Atta and Mouneir, 2004; Diehl et al., 2004). Owing to the support of national and international organizations for the studies on treatment and prevention of diarrhoeal diseases based on traditional practices, medicinal plants are becoming hopeful source of antidiarrhoeal drugs (Mukherjee et al., 1998; Tona et al., 1998; Otshudi et al., 2000a; Lin et al., 2002).

The people of India have a very long-standing tradition in the use of natural medicines and the local practices are still quite common in the treatment of diseases (Srinivasan et al., 2001; Harsha et al., 2002). The assessment of plants used in conventional medicines is anticipated to make available new antimicrobial agents (Otshudi et al., 2000b; Ryu et al., 2004). Thus, present study is aimed to investigate and establish the antidiarrhoeal potential of plant extracts used in folk medicine.

## 2. MATERIALS AND METHODS

### 2.1. Medicinal plant materials

Different ingredients (plant materials) were collected from their natural habitat (semi-arid regions of India), dried under shade and finally powdered using domestic grinder. The identity of plants was verified by the taxonomist at Botanical Survey of India, Arid Zone Circle, Jodhpur (India). Before the extraction, raw materials were pre-checked for pesticidal contaminations using suitable testing methods i.e. USP methods with GC-MS (data not given).

### 2.2. Preparation of crude extracts

#### 2.2.1. Preparation of water extract (decoction)

Individual ingredient (20g) were subjected to boil in 200 ml doubled distilled water in a 500 ml flask till the total volume remained one fourth. The water extract was filtered through a 420 ìm stainless steel filter, cooled and transferred to screw caped glass vials.

#### 2.2.2. Organic solvent extraction

Ingredients (10g) were extracted with the solvents of different polarities (methanol, chloroform and petroleum ether) by cold maceration for 24 hrs. The extracts were filtered through Whatman No. 1 filter paper, which was impregnated with same solvent. The organic solvents were concentrated to near dryness using rotary evaporator bath under reduced pressure. The extracts were further diluted with Dimethylsulphoxide (DMSO) for experimentation.

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### 2.3. Tested bacterial strain

Bacterial strain Enteropathogenic *Escherichia coli* (EPEC) was selected as it is the most common pathogen, which cause diarrhoea. This attaches to and damages the small intestine (Laven, 2002). The pathogen was cultured on nutrient agar media (HiMedia MM012). Extracts of all the herbal ingredients were screened against *Escherichia coli*. Bacterial strain was procured from patient suffering from gastrointestinal problem. All the chemicals were of analytical reagent grade (E-Merck).

### 2.4. Determination of antibacterial activity

#### 2.4.1. Preparation of inoculums

Bacteria were grown to lag phase before inoculated in to liquid broth medium for activation, the composition of which is as per the Indian Pharmacopoeia (1996) and incubated for 24 hours (Dykes et al., 2003). For all the experiments 0.1 ml cultures were inoculated in 10 ml broths giving final cell load of $10^6$ - $10^7$ CFU/ml in nutrient broth media (Musumeci et al., 2003; Sohn et al., 2004).

#### 2.4.2. Testing of antibacterial activity

#### 2.4.2.1. Agar well diffusion assay method

A 0.5ml volume of the standard inoculum ($10^6$-$10^7$ CFU) of the test bacterial strain was spread on Mueller Hinton Agar (MHA) with a sterile bent glass rod spreader and allowed to dry. Then, 6 mm-diameter wells were bored in the MHA. Plant extracts (1000, 500, 100 and 50 ì g/ml concentration) were introduced into each well and allowed to stand for 1 h at room temperature to diffuse before incubation at 37ºC for 24 h. The inhibition zone diameter (IZD) was measured by antibiotic zone reader to nearest mm (Okoli and Iroegbu, 2004).

#### 2.4.2.2. Total plate count agar method

To determine the number of the viable bacteria, 0.1 ml of the suspension mixture from plant extracts and bacteria were used for re-plate on Muller-Hinton agar plates. The samples were diluted with 0.85% normal saline solution to an appropriate concentration which gave a countable number of the colonies/plate. 0.1 ml of the diluted samples were spread on Muller-Hinton agar plate and incubated further for 18-24 h. at 37ºC in biological incubator (Wongkham et al., 2001).

#### 2.4.3. Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations (MICs) were determined using agar well diffusion assay method as described by Mendoza (1998).

## 3. RESULTS AND DISCUSSION

The results for the antimicrobial activities of different plant extracts in agar well diffusion assay method and total plate count agar method are presented in Table 1 and 2 respectively. In agar well diffusion assay method (Table 1), methanol extracts of *A. catechu, A. nilotica, A. marmelos, T. ammi* and *H. antidysenterica;* chloroform extracts of *A. catechu, A. indica* and *T. ammi,* and petroleum ether extracts of *A. marmelos, A. indica,* and *T. ammi* showed highest inhibition and the activities were reported at the concentration of 50 ì g/ml. Chloroform extracts of *A. nilotica, A. marmelos* and *H. antidysenterica,* and petroleum ether extract of *O. basilicum* were found active at the concentration of 100 ì g/ml. Aqueous and petroleum ether extracts of *A. nilotica;* chloroform and petroleum ether extracts of *A. squamosa,* and methanol and chloroform extracts of *O. basilicum* inhibited the pathogen at the concentration of 500 ì g/ml, while, little inhibition at the concentration of 1000 ì g/ml was also shown by methanol extract of *H. antidysenterica.* No visible growth inhibition of *E. coli* was observed during the screening of rest of the extracts at any of the studied concentration.

Results (Table 2) of antimicrobial activity of the different extracts at a concentration of 1000 ì g/ml using total plate count agar method revealed a high inhibition of *E. coli* growth by methanol extracts of *A. catechu, A. indica,* and *T. ammi;* chloroform extracts of *A. marmelos* and *A. indica,* and petroleum ether extracts of *A. indica* and *O. basilicum.* Moderate inhibition in the growth of *E. coli* by methanol extracts of *A. nilotica, H. antidysenterica* and *O. basilicum*; chloroform extract of *O. basilicum,* and petroleum ether extracts of *A. marmelos* and *H. antidysenterica* was observed. Little inhibition in the growth of pathogen was also observed in the plates containing aqueous extracts of *A. nilotica* and *O. basilicum;* methanol extract of *A. squamosa;* chloroform extracts of *A. nilotica, T. ammi* and *H. antidysenterica,* and petroleum ether extract of *T. ammi.* Rest of the extracts showed no inhibition in microbial growth, when screened against studied strain of *E. coli*.

Plants used in traditional Indian system of medicine have been found active against a wide variety of microorganisms (Khan et al., 1994; Ahmad et al., 1998; Ahmad and Beg, 2001). Many biochemical constituents of plants have been shown to possess excellent biological activities (Gupta et al., 1993; Cowan, 1999; Iwu et al., 1999; Ogunleye and Ibitoye, 2003; Tshikalange et al., 2005). Although, the reports on the use of studied plants viz. *Acacia catechu* (Felter and Lloyd, 1998), *Acacia nilotica* (Sawhney et al., 1978; Agunu et al., 2005), *Aegle marmelos* (Shrivastava, 1985; Dhuley, 2003; Rao et al, 2003; Mazumder et al., 2006), *Annona squamosa* (Santos and Santana, 2001), *Azadirachta indica* (Singh, 1986; Bhattarai, 1993), *Trachyspermum ammi* (Devasankaraiah et al., 1974; Singh, 1986), *Holarrhena antidysenterica* (Kavitha et al., 2004) and *Ocimum basilicum* (Geeta et al., 2001) for the treatment of diarrhoea are available but we found contradictory and equivocal reports on screening of their extracts against *E. coli* in the literature. The aqueous and ethanolic extracts of *Acacia catechu* and *Holarrhena antidysenterica* (Voravuthikunchai et al., 2004), *Acacia nilotica* (Jain et al., 1987), *Azadirachta indica* (Talwar et al., 1997), *Trachyspermum ammi* (Ray and Majumdar, 1976; Damle and Tipnis, 1980); chloroform extract of *Aegle marmelos* (Mazumder et al., 2006) and *Annona squamosa* (Adoum et al., 1997); seed oil (0.4%) of *Aegle marmelos* (Singh et al., 1983); and essential oil of *Ocimum basilicum* (Farouk et al., 1983; Janssen et al., 1986; Srinivasan et al., 2001) demonstrated antibacterial activities, while, aqueous

**Table 1:** Antimicrobial activity of plant extracts against *Escherichia coli* agar well diffusion assay method (n=3)

| Plant name | Extract type | | | |
|---|---|---|---|---|
| | Aqueous* | Methanol* | Chloroform* | Petroleum ether* |
| *Acacia catechu* | -- (30) | ++++ (60) | ++++ (320) | -- (>1000) |
| *Acacia nilotica* | ++ (120) | ++++ (70) | +++ (200) | ++ (140) |
| *Aegle marmelos* | -- (>1000) | ++++ (<50) | +++ (160) | ++++ (100) |
| *Azadirachta indica* | -- (>1000) | -- (>1000) | ++++ (30) | ++++ (110) |
| *Annona squamosa* | -- (>1000) | -- (>1000) | ++ (220) | ++ (160) |
| *Trachyspermum ammi* | -- (>1000) | ++++ (70) | ++++ (40) | ++++ (240) |
| *Holarrhena antidysenterica* | -- (>1000) | + (180) | + (>1000) | ++++ (50) |
| *Ocimum basilicum* | -- (>1000) | ++ (100) | ++ (70) | +++ (80) |

-, No inhibition; ++++, Inhibition at 50 ì g/ml; +++, Inhibition at 100 ì g/ml; ++, Inhibition at 500 ìg/ml; +, Inhibition at 1000 ì g/ml. *The different value of MIC in ì g/ml; is given in the parenthesis.

**Table 2:** Plate count of bacteria incubated in agar from the broth containing plant extracts for 24 hrs (n=3)

| Plant name | Extract type | | | |
|---|---|---|---|---|
| | Aqueous | Methanol | Chloroform | Petroleum ether |
| *Acacia catechu* | ++++ | + | ++++ | ++++ |
| *Acacia nilotica* | +++ | ++ | +++ | ++++ |
| *Aegle marmelos* | ++++ | ++++ | + | ++ |
| *Azadirachta indica* | ++++ | + | + | + |
| *Annona squamosa* | ++++ | +++ | ++++ | ++++ |
| *Trachyspermum ammi* | ++++ | + | +++ | +++ |
| *Holarrhena antidysenterica* | ++++ | ++ | +++ | ++ |
| *Ocimum basilicum* | +++ | ++ | ++ | + |

++++, > 500 X $10^5$ CFU/ml; +++, 200-500 X $10^5$ CFU/ml; ++, 50-200 X $10^5$ CFU/ml; +, < 50 X $10^5$ CFU/ml; -, No growth

extracts of all the studied plants reported equivocal and/or inactive by various researchers (Gupta et al., 1993; Ahmad et al., 1998; Chariandy et al., 1999; Ali et al., 2001). Alzoreky and Nakahara (2003) reported acetone and buffered methanol extracts of *Azadirachta indica* and *Ocimum basilicum* inactive against *E. coli*. We also got similar and/or contrast results with the findings of earlier research.

In present study, one or more organic extracts of each plant except *A. squamosa* were able to inhibit the growth of tested standard strain of pathogen to a certain extent at 100 ì g/ml that corresponds to 50-200 x$10^5$ CFU/ml in total plate count agar method. In case of *A. squamosa*, inhibition of microbial growth was recorded at 500 ì g/ml concentration. The highest activity was shown by methanol extracts of *A. nilotica* and *T. ammi*, and petroleum ether extract of *A. indica.* A number of explanations can be given for the difference in biological activity reports of some common extracts against same or similar microorganism, but the first logic is dissimilarities in phytochemicals of similar plants growing at different geographical locations (Olila et al., 2001). In the findings, there were marked differences at few places in the activities of some extracts in two antimicrobial testing methods. The variation in results during the antimicrobial efficacy in different testing methods of a compound transpires because of effect of medium and supplements (Jones, 1996), temperature and other inoculation conditions (Michel and Blanc, 1994), molecular weight and diffusion rate of compound through medium (Marshall et al., 1999; Olila et al., 2001) etc.

The results of present study indicate that plant extracts showing positive microbial activity provide the scientific base to include the traditional practices in modern system of medicines. They may, therefore, provide new leads in the development of new antimicrobial drugs for the therapy of diarrhoea and other infectious diseases caused by *E. coli*.

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