

## Research Article



## Phytal Extracts as Traditional Medicines to Control Gastroenteric Disorders in Humans

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## ABSTRACT

India has rich biodiversity and plants have been a source to cure different health problems from ancient times. Enteric problems such as diarrhea are caused by unhygienic eating habits and indigestion which often cause stomach pain, bloating, vomiting/loose or watery stools and food poisoning. Several bacteria and protozoan are the main causes. Imbalances in the natural concentration of the bacterial populations or toxins released by the microbes in the intestine effect the functioning of enteric system. Enterotoxins alter the water absorption levels resulting in diarrhea. Various home remedies like Ajwain/Coffee/Bael and Pomegranate have been used as traditional medicines in different parts of India to control the digestive disorders. Aqueous extracts of the above plants prepared as traditional medicines were assessed to check their efficacy during the study. The extracts of Bael leaves / peel of pomegranate are found to be effective in controlling enteric problems.

**Keywords:** Traditional medicine, phytal extracts, diarrhea, Ajwain, bael leaves, coffee, pomegranate.

## INTRODUCTION

Diarrhea one of the most common disorders, results due to several reasons such as unhygienic conditions of food preparation, water borne infections, food allergies or food poisoning. Diarrhea is defined as an abrupt increase in number of bowel movements and fluid loss (resulting from bacterial toxins) or excess salts and fats (from foods) into the lumen. Organisms such as enteric bacteria and viruses may possess the ability to release enterotoxins, such as cholera toxin, or an inflammatory response which can cause diarrhea<sup>1</sup>. The mode of action could be adherence of the organism to the wall of intestine or the toxins that bind to the plasma membrane causing changes / blocks in the cell signal phenomenon resulting in diarrhea and dysentery. Enteric bacteria causing watery or secretory stool includes *Vibrio cholera*, *ETECs* (Enterotoxigenic *E. coli*), *EPECs* (Enteropathogenic *E. coli*). Those causing bloody diarrhea and dysentery include *EIECs* (Enteroinvasive *E. coli*), *Shigella spp* (produces Shiga toxin) *Salmonella spp*, *EHECs* (Enterohemorrhagic *E. coli*). Some are included in slow pathogenic bacterial infection viz. *Helicobacter pylori*. *Salmonella spp* are found in undercooked chicken eggs and dairy products. *EHECs* (Enterohemorrhagic *E. coli*) is found in undercooked hamburger that produces Shiga toxin.

Age old traditional medicinal practices have used plants for controlling these enteric disorders in India. These practices are easy to handle, easy to manage and are effective if the symptoms are not chronic. Practices vary from place to place but, with a common target of controlling enteric problems. Different studies were done using the plant extracts of polar and non polar solvents such as ethanol, methanol extracts<sup>2,4</sup> and their

effectiveness to control pathogenic microbial cultures and enteric pathogens / castor oil based diarrheal conditions induced in to mice<sup>5,6</sup>. The current study is to examine efficacy of these decoctions used as household remedies for enteric disorders.

## MATERIALS AND METHODS

Different plants - bael (*Aegle marmelos L*, Family-Rutaceae), pomegranate (*Punica granatum L*, Family-Punicaceae), coffee (*Coffea arabica*, Family-Rubiaceae) and ajwain (*Trachyspermum ammi L*, Family-Apiaceae) were considered for current study. Plant materials were collected at local market and authenticated by Dr. Shashirekha, Botany department, Mithibai College, Mumbai. Fresh samples of bael leaves, rind of pomegranate, coffee seeds, and ajwain seeds were used to prepare decoctions with water.

## Preparations of traditional medicines

The preparations were made as per known practices of preparing traditional household decoctions.

Bael leaf extract: 5gm of fresh leaves were taken and homogenized using a mortar and pestel. 100 ml of distilled water was added to the homogenate and boiled for 10 minutes. The decoctions was cooled and filtered with a muslin cloth.

Pomegranate extract: 5 gm of the rind of pomegranate fruit was taken and homogenized using mortar and pestle and boiled in 100 ml of distilled water for 10 min. The decoction was cooled and filtered to use for checking anti diarrheal activity.

Coffee seeds: 5 gm of coffee seeds were roasted and ground into paste. 100 ml of hot boiled water was added



to the powder and heated for 10 minutes. Decoction was collected using a filter.

Ajwain extract: 5 gm seeds of Ajwain were roasted till they turned black and 100ml water was added and boiled for 10 min. The decoction was collected using a filter.

The decoctions were filtered and centrifuged at 2500 rpm for 10 minutes. The supernatant was checked biochemically for their phytochemical constituents as well as chromatography techniques<sup>7</sup>. The presence of phytochemicals such as tannins, alkaloids, steroid glycosides, flavonoids, saponins and anthracenoides were qualitatively determined from the decoctions using standard methods<sup>7</sup> and were analyzed.

The decoctions' inhibitory activity was tested using agar well diffusion method. Nutrient agar and Muller and Hinton agar media were used during the study. The microorganism cultures (a) available in the laboratory and (b) the ATCC cultures which cause diarrhea were considered to assess the efficacy of extracts. The results were compared among the different traditional methods to control diarrhea and, those reported in the previous studies.

### Bacterial Cultures

The standard pathogenic bacteria cultures were procured and used along with the normal laboratory cultures in the present study. The bacteria rejuvenated on Nutrient agar slants at 37°C for 18 h. Subcultures were prepared from the stock for bioassay. The size of inoculum of the bacterial culture was standardized according to the National committee for Clinical Laboratory Standards<sup>8</sup> guidelines. The pathogenic bacteria culture was inoculated into sterile nutrient broth and incubated at 37°C for 3 h until the culture attained a turbidity of 0.5 McFarland units. The final inoculum size was standardized to 10<sup>5</sup> CFU/mL with the help of SPC and Nephlo-turbidometer. 15 different strains of seven genera such as *Shigella spp.*, *Pseudomonas aeruginosa*, *P. aeruginosa* (ATCC 27853), *Salmonella typhi*, *S. paratyphi*, *Staphylococcus aureus*, *S. aureus* (ATCC 25923), *Escherichia coli*, *E. coli* (ATCC 25922), *E. coli* (ATCC 35210), *Enterococcus faecalis* (ATCC 51299), *E. faecalis* (ATCC 29212); *MRSA* (Methicillin-resistant *Staphylococcus aureus*); *Klebsiella pneumoniae* (ATCC 700603) and *Methicillin resistant Staphylococcus species* (MRSS) were considered for the sensitivity assessment against the traditional three extracts which were procured from DR. Ajay Shah Pathology Laboratory And Microbiology reference centre.

### Antimicrobial assay

The agar well diffusion method<sup>9</sup> whose modified version adopted<sup>10</sup> earlier was used. 0.1 ml of inoculums (10<sup>5</sup> CFU/ml) of selected test organisms was spread on MH agar plates and NA plates using a sterile cotton swab in order to obtain uniform microbial growth. Wells of 8mm diameter were punched into the agar medium. 25µl of

each extract (50mg/1ml concentration) was dispensed into the cup using micro-pipette fixed with sterile tips. Distilled water was used as negative control. The plates were incubated for 18h at 37°C. The antibacterial activity was evaluated and diameter of inhibition zones were measured in mm. Experiments were carried out in triplicate and the averages diameters of zone of inhibition were recorded.

The antibacterial activity was assessed in terms of Percent Overall Inhibition Efficiency Score (POIES) and Percent Overall Screening Efficiency Score (POSES)<sup>11</sup>. Since multiple microbial species were used in the test panel, the overall inhibition score for each individual decoction should be calculated.

The percent overall inhibition efficiency score (POIES) was calculated as total score for each decoction. POIES= (TNIS/TNTS) X100. Where, TNIS is total number of inhibited species and TNTS total number of test species. The ideal score for multispecific inhibition would be 100.

Percent overall screening efficiency score (POSES): The total no of positive results of each test pathogen against the controls that are used. POSES= (TPR/TAS) X 100, where TPR is total no of positive results of each test pathogen and TAS is total no of decoctions used for computation.

## RESULTS AND DISCUSSION

The antimicrobial studies of the four traditional extracts revealed different levels of sensitivity with respect to different microbial cultures. *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 35210) and *Pseudomonas aeruginosa* (ATCC 27853) showed resistance to the three medicinal decoctions such as Ajwain, bael and coffee. Inhibitory zones >20mm were observed for Pomegranate decoction in case of *Salmonella typhi*, *S. paratyphi*, *Shigella spp.*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* (ATCC 25923), *Escherichia coli*, *MRSA* (Methicillin-resistant *Staphylococcus aureus*); and *Methicillin resistant Staphylococcus species* (MRSS) and, *Pseudomonas aeruginosa* in the case of bael leaf decoction. Ajwain decoction inhibited seven bacterial species, coffee decoction five bacterial species followed by Pomegranate and bael two each with inhibitory zones in the range of 10mm -20mm. (Table-1). However, more number of species (5 species each) exhibited resistance for coffee, ajwain and bael decoctions. Sensitivity in the form of fuzzy zones with inhibition was observed for pomegranate in the case of *E. coli* (ATCC 25922) and *Enterococcus faecalis* (ATCC 51299) for the three decoctions other than ajwain. These results were not considered for sensitivity assessments.

The level of control in the form of inhibitory zone indicates the efficiency of phytal decoctions on the pathogenic bacteria that cause diarrhea. The percent overall inhibition efficiency score (POIES) and Percent Overall Screening Efficiency Score (POSES) used as efficiency parameters indicated the levels of controls of



these homemade medicines. Pomegranate decoction (66.6%) indicated higher levels of efficiency in controlling pathogens. Maximum control of pathogens was noticed in the case of *S. typhii* and *P.aeruginosa*, *S.paratyphii*, *Shigella spp.*, *S aureus*, *E.coli*, and *E.fecalis* (75%).

However, a detailed comparison of the current study with those of earlier studies indicated need to study the applicability of homemade decoctions as traditional medicines and their reliability.

**Table 1:** Phytal extracts as traditional medicine efficacy on microbial populations

Test Organisms	Coffee (mm)	Pomegranate (mm)	Ajwain (mm)	Bael (mm)	POSES
<i>S. paratyphi</i> .	16	31	10	R	100
<i>Shigella spp.</i>	10	20	12	R	75
<i>Staphylococcus aureus</i>	R	15	12	15	75
<i>S. aureus</i> (ATCC 25923)	R	20	R	R	75
<i>Pseudomonas aeruginosa</i>	10	26	14	20	25
<i>P. aeruginosa</i> (ATCC 27853)	R	R	R	R	100
<i>Escherichia coli</i>	12	22	15	R	0
<i>E. coli</i> (ATCC 25922)	R	S	12	R	75
<i>E. coli</i> (ATCC 35210)	R	R	R	R	25
<i>Enterococcus faecalis</i> (ATCC 51299)	S	S	R	S	0
<i>E. fecalis</i> (ATCC 29212)	R	R	R	R	0
MRSA (Methicillin-resistant <i>S. aureus</i> )	R	30	R	R	0
<i>Klebsiella pneumonia</i> (ATCC 700603)	R	12	10	R	25
Methicillin resistant <i>Staphylococcus species</i> (MRSS)	R	20	R	R	50
<b>POIES (Percent Overall Inhibition Efficiency Score)</b>	<b>33.3</b>	<b>66.6</b>	<b>53.3</b>	<b>20</b>	<b>25</b>

(Clearance zone around the well). (S: fuzzy, no clear inhibition zone, but the organism showed sensitivity towards the decoction.)

\*POSES: Percent Overall Screening Efficiency Score.

The compositions of homemade traditional medicinal decoctions were analyzed using biochemical reactions and thin layer chromatography. Phenols, Flavones, Carbohydrates, Saponins, Alkaloids were observed in coffee, while alkaloids were absent in Pomogranate, bael and Ajwain extractions. Saponins were absent in bael as well as Ajwain. (Table-2)

**Table 2:** Phytal extracts used as traditional medicine and Characterization:

Components	Ajwain	Pomegranate	Bael	Coffee
Phenolics	+	+	+	+
Flavanol	+	+	+	+
Carbohydrates	-	+	+	+
Saponins	-	+	-	+
Alkaloids	-	-	-	+

Key: - + Present - absent

Earlier study on Pomegranate fruit peel methanolic extract showed broad spectrum activity against *Bacillus subtilis* and *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia* at a conc. Of 0.2 to 0.78mg/ml and contained Polyphenol compounds such as ellagic tannins, flavonols, anthocyanins, catechin, procyanidins, ellagic acid and gallic acid have been implicated in various pharmacological activities in the fruit peel<sup>2</sup>. Pomegranate

peel also indicated the presence of phytochemical compounds such as mainly carbohydrates, glycosides, saponins, phenolic compounds, volatile oil (thymol,  $\gamma$ -terpinene, para-cymene, and  $\alpha$ - and  $\beta$ -pinene), protein, fat, fiber and mineral matter containing calcium, phosphorous, iron and nicotinic acid<sup>12</sup>. The aqueous extract of *Punica granatum* peels exhibited control over concentration dependent inhibition of diarrhea in the case of castor oil induced diarrhea in rats and protected rats against castor oil-induced diarrhea enteropooling<sup>5</sup> as well as multidrug resistant strains of Enteroaggregative *Escherichia coli* (EAEC)<sup>6</sup> as well as metronidazole drug resistant *Helicobacter pylori*, *S. aureus* and *P. aeruginosa*<sup>13</sup>.

Earlier antimicrobial studies with respect to ajwain (*T.ammi*) indicated that the antimicrobial actions were due to the active principles such as carvacol and thymol<sup>14</sup>. Thymol kills bacteria which can resist third generation antibiotics and multi-drug resistant microbial pathogens and thus works as a plant based 4<sup>th</sup> generation herbal antibiotic formulation<sup>15</sup>. Phenolic compounds, such as thymol and carvacol, are known to be either bactericidal or bacteriostatic agents depending on the concentration used<sup>12</sup>.

Most of the *Aegle marmelos* studies are associated with the hot aqueous extract (decoction) of dried unripe fruit pulp for its antimicrobial activity and effect on various

aspects of pathogenicity of infectious diarrhea<sup>16</sup>. Research on biological activities of crude extracts and their chemical constituents of bael leaves revealed the presence of Skimmarinine, Aeglin, Lupeol, Cineole, Cuminaldehyde, Eugenol, and Marmesinium; of which, Cuminaldehyde and Eugenol are antibacterial in nature<sup>17</sup>. The soxhelt ethanolic extraction of dried powdered bael leaves inhibited the bacterial species *S. aureus* which can cause travelers' diarrhoea<sup>18</sup>.

The overall results indicated that traditionally prepared Pomegranate decoction found to be more effective while inhibiting the microorganisms that can cause diarrhea followed by ajwain, coffee, and bael. The percent overall inhibition efficiency score (POIES) indicated higher level for Pomegranate decoction (66.6%) followed by ajwain (53.3%), coffee and bael decoctions. *S. typhii* and *P.aeruginosa* exhibited maximum Percent Overall Screening Efficiency Score (POSES) of 100%, followed by *S.paratyphii*, *Shigella spp.*, *S aureus*, *E.coli*, and *E.fecalis* (75%) and, *E.coli* and *K.pneumoniae* (50%). The POSES of *P.aeruginosa* (ATCC 27853), *E.fecalis* (ATCC 51299 and 29212) and *E.coli* (ATCC 35210) found to be zero while the Screening efficiency remained 25% for MRSA, MRSS and *S. aureus*.

## CONCLUSION

Diarrhea is a common disease which causes abdominal pain, motions, dehydration and may be deadly, especially in developing countries. The traditional usage of seven plant species viz., *Diospyros peregrina*, *Heritiera littoralis*, *Ixora coccinea*, *Pongamia pinnata*, *Rhizophora mucronata*, *Xylocarpus granatum*, and *Xylocarpus moluccensis* against diarrhea in Bangladesh has been well documented<sup>19</sup>. Several plant species like ajwain, coffee, pomegranate and bael are used as traditional medicines in different parts of India for the control of diarrhea. The decoction of the phytal extract is more efficient, handy to use, easy to prepare and has less or no side effects. Decoction is found to be effective against broad range of pathogens causing enteric diseases. The reason could be that Phyto chemicals have capability to alter the microenvironments of the gut. For e.g., tannic acid which chelates iron, prevents iron-seeking bacteria from reaching critical levels of growth; Polyphenols quench free radicals, inactivate carcinogens, stimulate cellular antioxidant defenses and change microenvironments<sup>20,21</sup>. Phytochemicals also alter the ionic concentrations in the microenvironment and disrupt communication and signaling pathways between the host and bacteria, thereby preventing or decreasing the severity of infection<sup>1</sup>. The crude extracts contain several compounds acting on different mechanisms<sup>22</sup> or the interplay of the constituents in the crude extract may result in better activity due to synergism or lead to decrease in toxicity and it is possible that pure compound(s) may not necessarily behave in the same manner as the natural extract<sup>16</sup>.

The preliminary screening of phytal decoctions for phytochemical constituents, their efficacy while inhibiting the pathogenic bacteria and efficiency score indicated that pomegranate is more effective followed by ajwain. Combined decoction of rind of pomegranate and bael was found to have antagonistic effect against laboratory organisms. Rind of Pomegranate is more effective against enteric bacteria as compared to bael. Studies are required to check the combination of these decoctions and the reason for their synergistic control or antagonistic control on the enteric bacteria. Detailed study on the presence of biologically active principles that may be active against diarrhea should be considered.

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