Haryana Vet. (June, 2020) **59(1),** 92-94 **Research Article**

IN VITRO EVALUATION OF ANTIMICROBIAL ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *ROSMARINUS OFFICINALIS* AND *CURCUMA LONGA* ON *ESCHERICHIA COLI* ASSOCIATED WITH DIARRHEA IN CHILDREN

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ABSTRACT

The present study was carried out to evaluate *in-vitro* antimicrobial activity of hydroalcoholic extract of *Rosmarinus officinalis* and *Curcuma longa* on *Escherichia coli* associated with diarrhea in children. In this experimental study, hydroalcoholic extract of Rosemary plant (*Rosmarinus officinalis*) and turmeric plant (*Curcuma longa*) with concentrations of 400, 200, 100, 50 and 25mg/ml were prepared and antimicrobial effects were analyzed. In order to study this effect, agar well diffusion was used and MIC and MBC determined. The results showed that the mentioned extracts were able to inhibit the growth of *E. coli* causing diarrhea in children, so these plants and their alcoholic extract can be used as alternate to antibiotics.

Keywords: Antibacterial, Children's diarrhea, *Curcuma longa*, *Escherichia coli*, Herbal extracts, MBC, MIC, *Rosmarinus officinalis*

Bacteria, viruses and parasites are the main cause of diarrhea in children despite advanced medical discoveries. The published statistical data by World Health Organization indicates that the intestinal diseases including diarrhea in developing and under developing countries are one of the major causes of mortality in young children and infants. Around one billion children are affected with diarrhea of age below 5 years and approximately 4.5 million die due to this problem. In Iran, diarrhea is the second cause of children's death after respiratory infections. In addition to child mortality, it also causes other problems like malnutrition, physical and mental retardation (Behrman *et al.*, 2000; Seas *et al.*, 2000).

Escherichia coli is a gram negative, facultative anaerobic, rod-shaped and non sporulating bacteria of the family Enterobacteriaceae. The harmless strains are part of the colonic flora of humans as well as animals and play an important role in physiology of the digestive system. A large number of strains belonging to these species have been characterized in different categories due to virulence genetic makeup. *Escherichia coli* causing diarrhea are classified according to their pathogenic characteristics. Among them each group creates a different disease mechanism (Kaper *et al.*, 2004).

There are six categories of intestinal *E. coli* pathotypes implicated in diarrheal disease. *E. coli* (DEC) that is responsible for about 30 to 40 percent of diarrheas include: Enterotoxigenic *E. coli*; Enteropathogenic *E. coli*; Enteroaggregative *E. coli*; Enteroinvasive *E. coli*; Enterohemorragic *E. coli*; Diffusely adherent *E. coli* (Persing *et al.*, 2016). A century before, herbal medicine was an important part of prevalent medicine but gradually population growth and science advancement have reduced the consumption of medicinal plants in many cases. One of the unpleasant effects of chemical drugs along with its beneficial effects is drug resistance. Iranian traditional medicine with a history of several hundred years has a high capacity for the prevention and treatment of the diseases and it can solve many medical problems. Herbal extracts and their compounds due to known antibacterial effects are widely used in traditional medicine and can control the growth of pathogenic bacteria. So these can be a good option for solving antibiotic resistance problems. Secondary plant metabolites such as essential oils and flavonoids widely have antimicrobial, antibacterial, antifungal and antioxidant properties.

Rosmarinus officinalis commonly known as rosemary is a member of mint family (Lamiaceae). This plant is a rich source of antimicrobial phenol compounds against gram negative and gram positive bacteria. The results of one study showed that the essential oils of rosemary have a significant antimicrobial activity in a specific concentration range (Fu *et al.*, 2007; Wagnera and Ulrichmerzenich, 2009).

Turmeric (*Curcuma longa*) is a flowering plant of the ginger family (Zingiberaceae) which is native to Southeast Asia. This plant is not only a food additive but also an important compound in medical science which is used as anti-inflammatory, carminative, anti-parasite, arthritis and high cholesterol treatment, blood purification, antimicrobial and antioxidant compound and a medicine for chronic liver disease. Turmeric has been known for a long time due to its oily and yellow colorant curcumin, traditionally produced by its raw material (Sidhu *et al.*, 1998). Hence, this research was done with the aim that use

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of herbal medicine can become substitute of chemical medicine which is having some adverse effects like carcinogenic and allergic in some human and animals.

MATERIALS AND METHODS

Children's diarrheic stool samples from Shiraznamazi hospital were taken to the laboratory of microbiology at Azad Kazerun University. For enrichment of culture, Trypticase soy broth (TSB) of Difco company was used and the isolation was carried out on MacConkey agar (MAC) for 24 hours. To evaluate the lactose fermentation and identification of isolated bacteria, eosin methylene blue medium (EMB) of Merck company were used. In order to detect *E. coli*, Triple sugar iron (TSI), IMVIC, and urea tests were performed.

Extraction of plants was done by maceration method. First, the plants were dried in shade and powdered. At this stage, 20 g of powdered plant was weighed and transferred to erlenmeyer flask and then macerated by 600 cc of solvent which contains 300 cc distilled water and 300 cc ethanol 96% for 72 hours. The extract was then shaken, filtered and placed in rotary machine for 20 minutes in order to remove solvent from extract. The pure extracts were obtained in sterile containers and covered and then stored in refrigerator. Extracts were prepared at 400, 200, 100, 50, 25 mg/ml concentrations and dissolved in 5 % solvent (dimethyl sulfoxide) for agar well diffusion test and MIC determination. For agar well diffusion test, a culture of suspension with turbidity of 0.5 Mcfarland standards was made in Muller Hinton agar. The solvent itself was used as a negative control and chloramphenicol used as positive control. Then the microbial cultures were incubated at 37 °C for 24 hours. Finally the ruler was used for measurement of inhibition zones. McFarland standards are used as a reference to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a given range to standardize microbial testing. A sterile swab was used to transfer several colonies were to sterilized tube that contained physiological serum. After mixing the solution, with the help of the lights the turbidity was compared with the 0.5 McFarland turbidity.

MIC determination using micro dilution method

For this purpose, the bacterium was cultured 24 hours at 37 °C in Muller Hinton broth. Dilution extracts including 400, 200, 100, 50, 25 mg/ml were prepared. 100 μl were added to 96-well microplates that previously contained 100 μl of bacterial suspension with McFarland turbidity 0.5. Then similar test were carried out for positive control (a culture medium containing bacteria without extract) and negative control (a nonbacterial culture medium). The microplates were then incubated at 37 °C for 24 hours. Positive control well presents as opaque and most turbid.

As extracted concentration increases, the turbidity decreases until the MIC is reached. So the lowest concentration of extract with the least turbid reported as MIC (Minimum Inhibition Concentration). These experiments were repeated and averages were reported (Angioni *et al.*, 2004).

MBC determination

According to results of minimum inhibitory concentration, minimum bactericidal concentration was also determined. A loopful of broth from each test which was not showing growth was inoculated into Muller Hinton agar and incubated for 24 hours at 37 ºC. The lowest level of extract that resulted in microbial deaths was reported as MBC. Each experiment was repeated 3 times (Forbes *et al.*,1990).

RESULTS AND DISCUSSION

The results of various concentrations of hydroalcoholic extracts of *Rosmarinus officinalis* on inhibitory zone diameters are presented in Table 1. The results showed that hydroalcoholic extracts of *Rosmarinus officinalis* inhibits the growth of *E. coli* and as extract concentration increases, the diameter of the zone of inhibition also increased. The findings are in agreement with Deba *et al.* (2008) who reported essential oils of this plant have monoterpenes such as α-pinene, β-pinene, myrcene 1, 8-cineole and borneol as the major components. These compounds possess strong antibacterial and antimicrobial activities. The synthesis of silver nanoparticles by using rosemary aqueous extracts was successfully performed with the obtainment of synthesized particles with antibacterial potential against human pathogens (Ghaedi *et al.*, 2015). Sacco *et al.* (2015) assessed the antimicrobial effects of three rosemary ethanolic extracts (with distinct phenolic compositions) against *E. coli*, *P. aeruginosa* and *S. aureus* strains using a broth dilution method and reported that all extracts were more active against *E. coli*, being the extract rich in terpenoid derivatives, such as carnosic acid, which is known to present the best antibacterial activity.

The results of various concentrations of hydroalcoholic extracts of *Curcuma longa* on inhibitory zone diameters are presented in Table 2. Results showed that hydroalcoholic extracts of *Curcuma longa* inhibit the growth of *E. coli* and as extract concentration increases, the diameter of the zone of inhibition also increased. Curcumin from turmeric was renowned for its antibacterial activity through disruption of ion channels across the bacterial cell wall (Aly and Gumgumjee, 2011).

The results of minimum inhibitory and bactericidal concentration (mg/ml) of hydroalcoholic extracts of *Rosmarinus officinalis* and *Curcuma longa* are presented

Table 1

Inhibitory zone diameters of *Rosmarinus officinalis* (mm)

Concentrations mg/ml	25	50	100	200	400	Positive control	Negative control
Escherichia coli bacteria	—	—	7	12	16	22	—

Table 2

Inhibitory zone diameters of *Curcuma longa* (mm)

Concentrations mg/ml	25	50	100	200	400	Positive control	Negative control
Escherichia coli bacteria	—	3	9	14	17	24	—

in Table 3. The results indicated that hydroalcoholic extracts of *Rosmarinus officinalis* showed higher minimum inhibitory and bactericidal concentration as compared to hydroalcoholic extracts of *Curcuma longa* for *E. coli* (Table 3). Sacco *et al.* (2015) reported that the antimicrobial effects of three rosemary ethanolic extracts (with distinct phenolic compositions) against *E. coli*, *P. aeruginosa* and *S. aureus* strains using a broth dilution method. According to the authors, all tested extracts were more active against *E. coli* (MBC < 0.07 mg/mL), being the extract rich in terpenoid derivatives, such as carnosic acid, which is known to present the best antibacterial activity. Elsewhere, Basniwal *et al.* (2011) found that the curcumin nanoparticles are believed to manifest antibacterial properties by anchoring to the cell wall of the bacterial cell, breaking it, then penetrating inside the cell, and disrupting the structure of cell organelles.

It was concluded that *Rosmarinus officinalis* and *Curcuma longa* extracts had an inhibitory effect on *E. coli* bacteria. Rosemary extract has inhibitory effect at 100/ 200/400 mg/ml concentrations but *Curcuma longa* has this effect at 50 mg/ml concentration. Minimum inhibitory concentration for *Curcuma longa* extract is 50 mg/ml and for *Rosmarinus officinalis* extract is 100 mg/ml. Considering the antibacterial effects of the extracts and problem of drug resistance, use of these herbal compounds beside orthodox medicine treatments can be helpful.

Table 3

Minimum inhibitory and bactericidal concentration (mg/ml)

Escherichia coli bacteria	Minimum inhibitory concentration (MIC)	Minimum bactericidal concentration (MBC)
Rosmarinus officinalis hydroalcoholic extract	100	200
Curcuma longa hydroalcoholic extract	50	100

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