

**IN VITRO EVALUATION OF ANTIMICROBIAL ACTIVITY OF
ENTOBAN SYRUP; A POLYHERBAL FORMULATION**

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ABSTRACT

The global dilemma of antimicrobial resistance is predominantly pressing in developing countries, where the infectious disease burden is high and cost constraints put off the appliance of expensive agents. Although there are splendid advancements in modern medicine, yet traditional medicine has always been accomplished for treating infections of gastrointestinal tract. A polyherbal formulation Entoban integrates an outstanding combination of *Holarrhena antidysenterica*, *Berberis aristata*, *Symplocos racemosa*, *Querecus infectoria* and *Helicteres isora* used for the treatment of acute gastrointestinal infections. Therefore the present study was directed to evaluate the

invitro antimicrobial activity of polyherbal formulation against microorganism frequently involved in gastrointestinal infections. An antimicrobial activity was evaluated against five gram negative bacterial cultures namely *Salmonella enteric*, *Eschericia coli*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Vibrio cholera* and one gram positive bacterial culture *Staphylococcus aureus* by agar well diffusion method. The prepared syrup inhibited the growth of these organisms. Our findings suggest that, prepared polyherbal formulation have great potential against pathogenic microbes and can be used as antimicrobial agent for treatment of various infectious diseases of gastrointestinal tract.

KEYWORDS: Antimicrobial, Entoban, herbal, gastrointestinal infections.

INTRODUCTION

Among infectious diseases acute gastroenteritis remains a widespread complaint amongst infants and children all over the globe.^[1] Acute gastroenteritis is epitomize by diarrhea,

coupled with nausea, vomiting, fever, and abdominal pain.^[2] Most cases of mild diarrhea are of viral etiology, while severe diarrhea, especially associated with fever, tends to be of bacterial etiology. Chronic infectious diarrhea is often caused by parasites.^[3] Although there are splendid advancements in modern medicine, yet traditional medicine has always been accomplished for treating gastrointestinal infections. The traditional medicine sector has become an imperative resource of health care, particularly in rural and tribal areas of the country.^[4] Herbal remedies have shown remarkable success in healing acute as well as chronic diarrheal diseases.

The global dilemma of antimicrobial resistance is predominantly pressing in developing countries, where the infectious disease burden is elevated and cost constraints put off the appliance of expensive agents. Despite the existence of potent antibiotic and antifungal agents, resistant or multi-resistant strains are continuously appearing, imposing the need for a permanent search and development of new drugs.^[5] The less availability and high cost of new generation antibiotics necessitates looking for the substances from alternative medicines with claimed antimicrobial activity. Traditionally, plants based drugs have proven to be an excellent source of inspiration for novel drug compounds and extremely successful in the fight against microbial infections.^[6] The potential for developing antimicrobials into medicines appears rewarding, from both the perspective of drug development and the perspective of phytomedicines. Many commercially proven drugs used in modern medicine were initially used in crude form in traditional or folk healing practices, or for other purposes that suggested potentially useful biological activity. The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment. Entoban integrates an outstanding combination of *Holarrhena antidysenterica*, *Berberis aristata*, *Symplocos racemosa*, *Quercus infectoria* and *Helicteres isora* used for the treatment of acute gastrointestinal infections. Previous studies have demonstrated the antimicrobial activity of the ingredients present in the formulation.^[8-10] Therefore the present study was directed to evaluate the invitro antimicrobial activity of polyherbal formulation Entoban syrup against microorganism frequently involved in gastrointestinal infections.

MATERIAL AND METHOD

Apparatus used

Pre sterilized glass petri dishes, Metallic borer, volumetric flask, Pyrex A (Germany), Sanyo lab autoclave, MLS-3780, S.NO-2Y0301, Phase, Company Sanyo electric co, Ltd Made in Japan, Streamline Horizontal laminar flow cabinet, ESCO. En 1822.1 class H 13. HEPA filters, ISO 14644.1 Class 4. IEC 61010-1, US Federal standard 209E Class 10, Uni-Bloc SHIMADZU. Capacity Maximum 220 g and Minimum 10mg. SHIMADZU corporation made in Japan, Irmeco hybridization Oven. Digital constant temperature tank (China). Item-Model VRN-360. RPM 60-230 +10, Gemmy industrial corp (Taiwan).

Test Microorganism

Five gram negative bacterial cultures namely Salmonella enteric, Eschericia coli, Shigella dysenteriae, Pseudomonas aeruginosa, Vibrio cholera and one gram positive bacterial culture Staphylococcus aureus were used in this investigation. All the cultures were obtained from Dr Essa laboratories, Karachi, Pakistan.

Preparation of McFarland (0.5) Index

McFarland (0.5) index was prepared by adding 0.5 ml of 1.175% w/v barium chloride solution to 99.5ml of 1.0% sulphuric acid solution and mixed carefully. This index was matching to approximate bacterial cell density of 1.5×10^8 CFU/ml. The absorbance of this index was 0.136 as noted by spectrophotometer (Spekol 2000 series, Analytikjena). The solution was stored in screw capped test tubes at room temperature in dark and check absorbance after storage.

Preparation of Tryptic Soy Agar

Tryptic Soy Agar (TSA) medium provided the necessary nutrients to support the growth of the microorganisms tested and a suitable medium to perform susceptibility testing. Tryptic Soy Agar (TSA) was prepared as the method mentioned by manufacturer (OXOID, USA). TSA powder was dissolved in distilled water on water bath and then autoclaved.

Culture preparation

Overnight cultures were kept for 24 h at $36^\circ\text{C} \pm 1^\circ\text{C}$ and the purity of cultures was checked after 8 h of incubation. After 24 h of incubation, bacterial suspension (inoculum) was diluted with sterile physiological solution, to 10^8 CFU/mL.

Preparation of inoculum and standardization

The turbidity of inoculum corresponding bacterial cell density in TSA is an important factor which may affect the result interpretation of sensitivity test. McFarland Index (0.5) was used to standardize inoculated TSA.^[11] Adjustment in the turbidity of inoculated broth was done by noting absorbance using spectrophotometer.

Antimicrobial Activity Assay

The antimicrobial assay was performed by agar well diffusion method.^[12, 13] According to this method, 0.1 ml of diluted inoculums (10^8 CFU/ml) of test organism was thoroughly mixed with 20 ml of molten sterile TSA and poured in pre sterilized petri dishes under sterile condition. All plates were left to set at room temperature for 30-40 minutes. A well of 6mm diameter was made in the centre of each seeded plates by using sterile cork borer. Holes were then filled aseptically with 0.1 ml of Entoban syrup. Ciprofloxacin was used in comparison as a positive control. 1mg of ciprofloxacin was dissolved in 1ml of triple distilled water. Antibacterial plates were incubated at $37\pm 1^\circ\text{C}$ for 24 hours. The antibacterial activity was evaluated by measuring the zone of growth inhibition surrounding the well. The diameter of inhibition zone was measured in millimeters (mm) by vernier caliper.

RESULT AND DISCUSSION

Polyherbal formulation Entoban syrup was evaluated for its in vitro antimicrobial activity. Entoban integrates an excellent combination of herbs (Table 1) that have been used for decades to eliminate microorganisms and worms from gastrointestinal tract. It is the combination of *Holarrhena antidysenterica*, *Berberis aristata*, *Symplocos racemosa*, *Quercus infectoria* and *Helicteres isora*. Sucrose, a sweetening agent and main content of syrup, has been used to mask the bitter taste of the extract and so, a pleasantly tasting oral dosage form of the extract was formulated. Screening of anti-microbial activity was carried out by agar well diffusion method. An antimicrobial activity was evaluated against five gram negative bacterial cultures namely *Salmonella enteric*, *Eschericia coli*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Vibrio cholera* and one gram positive bacterial culture *Staphylococcus aureus*. (Table 2) The prepared syrup inhibited the growth of these organisms. It therefore indicates that the excipients and method of preparation did not affect the sensitivity of the active principles of the extract present in the formulation. Zone of inhibition of the developed formulation was comparable with the positive control. (Figure 1)

Herbal remedies have shown remarkable success in healing acute as well as chronic diarrheal diseases. *Holarrhena antidysenterica* Wall. is an important medicinal plant belonging to the class Apocynaceae. Research has shown that different parts of *H. antidysenterica* executed antibacterial activity. It is reported that bark of the plant showed anti diarrheal and astringent activity.^[14] The antimicrobial activity of *H. antidysenterica* bark extract has been reported against enteropathogens like enteroinvasive *Escherichia coli*, EPEC, *Salmonella typhimurium*, *Salmonella enteritidis*, *Shigella flexneri*, *Sh.boydii* and *Vibrio cholera*.^[8] *Berberis aristata* also shows wide antibacterial activity against Gram-positive bacteria and Gram-negative bacteria. The Gram-negative bacteria which are responsible for causing diarrhea and dysentery are susceptible to the extracts of *B. aristata*.^[9] The chloroform extracts of the root of *Aegle marmelos* (Correa) Linn. was found to be mostly active against the strains of *Vibrio cholerae*, followed by *Escherichia coli* and *Shigella* spp.^[10] The present work equally has confirmed such findings. Although Ahonkhai, et. al^[15] stated that application of heat in the extraction method may have affected the potency of the antifungal principle(s), this work has shown that application of heat to the various extracts did not affect the activity of the extract to bacterial organisms in formulation.

Table 1. Herbs used, quantity and pharmacological action in Entoban syrup

No.	Herbs ingredients	Common name/Parts	Quantity	Bioactivity (References)
1.	<i>Aegle marmelos</i>	Baeil/unripe dried fruit_dried pulp	Syrup; Oral; 100 mg / 10 ml	Antimicrobial activity and effect on various aspects of pathogenicity of infectious diarrhea, antioxidant, anti-microbial, gastrointestinal protective, antimicrobial, ulcer ^[16]
2.	<i>Berberis aristata</i>	Rasuat/small black fruit	Extract 30 mg/10ml	Gastrointestinal protective ^[9]
3.	<i>Butea frondosa</i>	Plas papra/gum_leaves	Dry extract 20mg	Acute diarrhea, chronic diarrhea, anti-inflammatory ^[17]
4.	<i>Holarrhena antidysenterica</i>	Kurchi/dried bark	Dry Extract 50mg/10ml	Antihelminthic, diarrhea ^[8]
5.	<i>Myrtus communis</i>	Habulas/Berries	Dry Extract 200 mg/10ml	Anti-inflammatory and antinociceptive ^[18]
6.	<i>Quecrus infectoria</i>	Majuphal/gall	Dry Extract 50 mg / 10 ml	Abdominal pain, antidiarrheal, antidysenterica, astringent agents, anti-inflammatory ^[19]

Table 2: Zone of Inhibition for formulation

Test Organisms	Diameter of zone of inhibition (in mm)					
	1	2	3	4	Mean \pm S.D (n= 4)	Positive control
Gram positive bacteria						
Staphylococcus aureus	19	17	18	19	18.25 \pm 0.957	23
Gram negative bacteria						
Salmonella enterica	24	21	21	22	22 \pm 1.438	26
Eschericia coli	19	19	20	18	19 \pm 0.816	23
Shigella dysenteriae	17	20	19	18	18.5 \pm 1.290	19
Pseudomonas aeruginosa	20	17	17	19	18.25 \pm 1.5	24
Vibrio cholerae	20	19	19	18	19 \pm 0.816	25

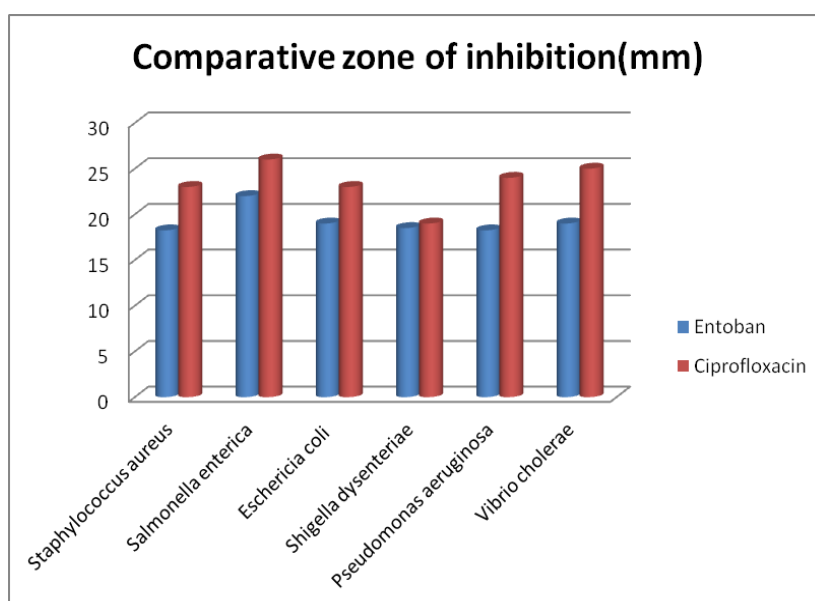


Figure 1: Comparative zone of inhibition of Entoban and ciprofloxacin

CONCLUSIONS

Our findings suggest that, prepared polyherbal formulation have great potential against pathogenic microbes and can be used as antimicrobial agent for treatment of various infectious diseases of gastrointestinal tract. The additives present in the formulation and the application of heat in the extraction method did not affect the antimicrobial activity.

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