

# Anti Entero Bacterial Activity of Hawthorn Berry Ethanolic Extract an *in vitro* study

Lakshmi.T\*<sup>1</sup> & Rajendran.R<sup>2</sup>

<sup>1</sup>Reader ,Department of Pharmacology ,Saveetha Dental College & Hospitals,Chennai.

<sup>2</sup>Green Chem Herbal Extracts & Formulations,Bangalore.

## Abstract

Enteric pathogens play a prominent role for causing health problems in most of the developing countries. These pathogens cause disease symptoms ranging from mild gastroenteritis to life threatening systemic infections and dehydrating diarrhea. Diarrhea genic organisms (*E. coli* , *S.typhi*,*S.boydii*,*V.cholerae*) are the most common bacterial pathogens implicated in diarrhea worldwide. To combat the spreading dreadful disease several researches have been done in plant extracts to prove its efficiency to treat diarrhea. My work is aimed to focus on the antibacterial activity of Hawthorn berry ethanolic extract (HBEE) against these enteric pathogens responsible for causing diarrhea.Macrobroth dilution method is employed in this study to find out Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC). Results concluded that HBEE showed significant antibacterial activity against enteric bacterial pathogens tested at different concentrations.

## Keywords

Enteric pathogens, diarrhea ,antibacterial ,hawthorn berry

## INTRODUCTION

Enteric or diarrheal infections are major public health problems in developing countries. The world health organization estimates that the diarrheal diseases, including those due to enteric pathogens,cause approximately 2.2 million deaths each year, primarily among infants & young children. Enteric bacteria comprised of *Salmonella* sp., *Shigella* spp., *Proteus* sp., *Klebsiella* sp., *Escherichia coli*, *Pseudomonas* sp., *Vibrio cholerae* and *Staphylococcus aureus* are the major etiological agents of sporadic and epidemic diarrhea both in children and adults.<sup>1</sup>

Bacterial resistance to antibiotics represents a serious problem for clinicians the widespread screening of medicinal plants from the traditional system of medicine seems to be safer and more effective agents that can be used to combat infectious diseases<sup>2</sup>. The traditional medical practitioners use a variety of herbal preparations to treat different kinds of diseases including microbial infections

Hawthorn (Family: *Rosaceae*) is a thorny tree that thrives in hedgerows and fields in the temperate regions of Europe and the British Isles. Hawthorn tea is most widely known for its medicinal uses and primarily for its cardiovascular applications. Hawthorn is rich in triterpenic acids like oleanolic acid and ursolic acid ; polyphenols like, epicatechin, procyanidin B2, procyanidin B5, procyanidin C1, hyperoside, isoquercitrin and chlorogenic acid.<sup>3</sup>

The flavonoid content, oligomeric proanthocyanidin possess good cardiotoxic property.The drug from flowers has anti-spasmodic, hypotensive, cardiotoxic, diuretic and nerve-sedative

Properties.<sup>4</sup>It is also utilized for their astringent qualities for relief of discomfort of sore throats, diarrhea and dysentery.

<sup>5,6</sup>The berries, leaves, and flowers of the hawthorn plant have been used for medicinal purposes. The berries contain more hyperoside than the leaves and flowers, and the leaves and

flowers contain more vitexin rhamnoside than the berries.<sup>7</sup>Hawthorn berries are more often used to make tinctures than teas, smoothies and punches. May also be taken encapsulated or as an extract.

Hawthorn herbal remedies also have an effective relaxing effect on the functioning of the nervous system, the herb aids in relieving excessive stress and anxiety, it helps in calming mental agitation, it lessens restlessness and reduces nervous palpitations. The herb also induces sleepiness in people affected by insomnia. The herbal remedies made from the hawthorn also have a diuretic action on the body.The herb is helpful to women in menopause, as it aids in removing debility or night sweats in those affected by them. The hawthorn berries can be made into an herbal decoction, which can be used as an astringent gargle for sore throats as well as an herbal douche for women affected by excessive vaginal discharges.<sup>8</sup>

Flavonoids are responsible for the red to blue colors of the hawthorn berries. Crataegus extracts also contain Cardiotoxic amines like phenylethylamine, tyramine, isobutylamine, and o-methoxyphenyl ethylamine ,Choline and acetylcholine ,Purine derivatives: adenosine, adenine, guanine and caffeic acid ,Amygdalin.<sup>9</sup>

Some people use hawthorn for digestive system complaints such as indigestion, diarrhea, and stomach pain.but there had been no literature supporting this statement.

Hence ,the present study was to aimed to evaluate the antibacterial activity of Hawthorn berry ethanolic extract(HBEE) on selected enteric pathogens in order to treat diarrhea.

## Plant material

Refined Hawthorn berry ethanolic extract (HBEE) is obtained as a gift sample from Green Chem herbal extract and formulations,Bangalore.

**Test microorganisms**

Bacterial pathogens [MHA-Hi media ,Mumbai]	ATCC
<i>S.boydii</i>	9027
<i>E.coli</i>	25922
<i>V.cholerae</i>	6395
<i>S.typhi</i>	19430

**METHODOLOGY****Medium used**

Tryptic soy broth is used as a medium

**Preparation of different concentrations of herbal extract**

The herbal extracts each 200mg were weighed aseptically into a sterile tube and dissolved in 2ml of sterile Tryptic soy Broth (TSB). From the stock solution various concentrations were prepared, viz., 0.15mg, 0.31 mg, 0.62 mg, 1.24mg, 2.5mg, 5mg, 10mg, 50mg, 100mg/100µl respectively in to wells of micro plates. The tested organism was grown in (TSB) Tryptic soy broth medium [MHA-Hi media ,Mumbai] for 24hrs at 37°C and concentration was adjusted to 0.5 Mac farland Standard.<sup>10-12</sup> The different concentrations of extracts were taken in 100µl quantities in a U bottom micro culture plates. Control well received plain broth without plant extract. The plates were kept in sealed covers and incubated at 37°C overnight and growth/no growth was detected. All the tests were done in triplicate to minimize the test error.

**Minimum Inhibitory Concentration (MIC)**

Minimum inhibitory concentration of herbal extracts against tested micro organisms was determined by macro broth dilution method.<sup>13</sup> A series of two- fold dilution of each extract ( 0.15mg/100µl to 100mg/100µl) was made in to which 100µl of the standardized bacterial suspension containing 10<sup>6</sup> organisms was made in Tryptic soy broth as specified by National Committee for Clinical Laboratory Standards (NCCLS, 1990).<sup>14</sup> The control well received plain broth without herbal extract .The plates were incubated at 37°C for 24 hours and observed for visible growth. As the extracts were colored, MIC could not be read directly by visual methods. Hence subcultures from all the wells were made and growth/no growth is detected. Then the MBC were obtained.

**Minimum Bactericidal Concentration (MBC)**

The MBCs were determined by selecting wells that showed no growth. The least concentration, at which no growth was observed, was noted as the MBC.

**RESULT AND DISCUSSION**

Use of natural medicinal products have become vital in view of their safety. A novel estimate suggests that, in many developing countries people depends on traditional practitioners and medicinal plants to meet primary health care needs. The present study was conducted to investigate antibacterial properties of Hawthorn berry ethanolic extract which is not studied and used in Indian Folkloric Medicine for treatment of diarrhea.

Herbal remedies play a prominent role in traditional medicine in rural areas of India where the therapeutic treatment of choice as antiseptic, anti-inflammatory and in treatment of infectious diseases including diarrhoea. In present study attempt was made to check the anti bacterial property of HBEE against enteric bacterial pathogens responsible for causing diarrhea.

Related to the Antibacterial screening it was observed that *E.coli* exhibited potent activity whereas the other organisms used in the test (*S.typhi*, *S.boydii*, *V.cholerae*) showed moderate antibacterial activity.

HBEE inhibited the growth of *E.coli* at 5mg/ml , *S.typhi* at 10mg/ml , *S.boydii* and *V.cholerae* at 50mg/ml respectively. No Growth indicates high effectiveness of the extract whereas presence of Growth indicates less effectiveness of the extract.

**CONCLUSION**

Results from this study showed the therapeutic activity of Hawthorn Berry Ethanolic Extract (HBEE) against some selected members of the entero bacteriaceae. This herb can therefore be used to manage enteric infections like diarrheal diseases. Toxicological studies, purification and identification of the plant active principles should be emphasized upon in addition to investigating its activity on a wider range of bacteria.

**Table 1: Anti-bacterial activity of HBEE against Enteric bacterial pathogens**

Bacterial pathogens	Concentration of HBEE (mg)									MBC
	0.15	0.31	0.62	1.24	2.5	5	10	50	100	
<i>E.coli</i>	G	G	G	G	G	NG	NG	NG	NG	5 mg/ml
<i>S.typhi</i>	G	G	G	G	G	G	NG	NG	NG	10mg/ml
<i>S.boydii</i>	G	G	G	G	G	G	G	NG	NG	50mg/ml
<i>V.cholerae</i>	G	G	G	G	G	G	G	NG	NG	50mg/ml

NG =No Growth indicates high effectiveness of the extract

G = Growth indicates less effectiveness of the extract

Hawthorn Berry ethanolic extract (HBEE)

- Inhibits the growth of *E.coli* at 5 mg/ml
- Inhibits the growth of *salmonella typhi* at 10 mg/ml
- Inhibits the growth of *Shigella boydii* at 50 mg/ml
- Inhibits the growth of *Vibrio cholerae* at 50 mg/ml

**CONFLICT OF INTEREST:** Nil

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