



THERAPEUTIC HERBAL ENEMA DEVICE FOR INTUSSUSCEPTION BY APPROACH OF IN-VITRO SUSCEPTIBILITY TEST

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ABSTRACT

Intussusception is a one portion of intestine invades into adjacent side. It is pediatric emergencies found in one to four in two thousand infants and children. The exact cause of intussusception is idiopathic among many different criteria bacterial intervention is one of the factors which are lead point for intussusception. The infection risk was found by *Salmonella species*, *Escherichia coli*, *Yersinia enterocolitica*, many more. To treat intussusception enema reduction and surgical intervention are possible methods to reduce intussusception. The new therapeutic herbal enema drug deliverable electronic device has been proposed in this paper, evaluation of antibacterial effect of combination leaves of the *Clitoria ternatea*, *Nyctanthes arbortristis*, and *Ocimum sanctum* are extracted using ethanol and methanol for herbal enema by Soxhlet extraction. The extracts of above are led to susceptibility test by in vitro analysis of four bacterial against *Salmonella typhimurium*, *Escherichia coli*, *Bacillus Subtilis* and *Enterobacter aerogens*. The extracts were tested in the duplicates. The extracted herbal solution possessed antibacterial activity which is confirmed by using in vitro approach of the Agar well diffusion method. The concentration of extracts with 10 μ l, 20 μ l and 50 μ l solutions of methanol and ethanol extract respectively are used for testing by considering tetracycline for standard result. Methanol and ethanol extract against *Salmonella typhimurium* exhibited the maximum inhibition zone up to 2.00cm, a dosage of 50 μ l. The sensitivity was found in both methanol and ethanol based solution. Compared to existing non-operative technique to reduce intussusception the proposed solutions will possess antibacterial activity and leaves have medicinal properties to cure intestinal problems. The proposed solution is a reliable, unsophisticated, and can be applied in Clinic's and Hospital's. The safe and secure device for reducing the intussusception for infantile has been proposed and referred for clinical trials and may lead to practical implementation.

Keywords: Intussusception, dried powder, Herbal solution, Ethanol extracts, Methanol extracts, Agar well Diffusion Method.

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INTRODUCTION

Intussusception is a pediatric emergency where one portion of intestine invades one portion into another portion. It occurs more frequently in infants than adults(1). Around up to 90% of pediatric intussusception causes are idiopathic and enema therapy is a prior choice. Bacteria intervention is a major cause of intussusception. The bacterial species namely *Salmonella species*, *Escherichia coli*, *Shigella species*, *Campylobacter* and *Yersinia enterocolitica* are the major bacteria which causes intussusception(2). The identification of intussusception by doctors is achieved by radiological methods.

Reduction of intussusception is achieved by operative and non-operative methods. Pneumatic reduction and hydrostatic reduction is performed safely by experienced radiologist and pediatric surgeon. By previous analysis both the methods are having successful rates. Failure in the above methods is referred for surgery. The symptoms of intussusception are rectal bleeding, abdominal pain, vomiting, diarrhea, distention and constipation(3).

The one of the major complication of pneumatic air reduction is bowel perforation and portal venous gas. Operative and non-operative reduction which are used there is a long history which support non-surgical reduction of the same(4). The demerit of the surgical method is patient has to undergo anesthesia. For infants it is a challenging task to give anesthesia. The non-operative technique is comparatively preferred to reduce intussusception. In adults surgical method is preferred for clearing intussusception(5). The repeated enema with particular delay is a better therapeutic solution for curing intussusception(6).

In this paper a new herbal enema passing automated device has been proposed and referred for clinical trials. The proposed solution is made up of leaves of *Clitoria ternatea*, *Nyctanthes arbor-tristis*, and *Ocimum sanctum*. Herbs are the human part of medicine from the time of civilization. They are the key sources of medicine. The herbal extract is obtained from Soxhlet extraction method. The above said leaves are therapeutic, antibiotic, safety and importance in making drugs. The (table 1) gives the detail taxonomy of plants profile(7-9).

Table 1: Taxonomy of traditional medicinal leaves

Species	<i>Clitoria ternatea</i>	<i>Nyctanthes arbor-tristis</i>	<i>Ocimum sanctum</i>
Kingdom	Plantae	Plantae	Plantae
Subkingdom	Viridaplanta	Viridiplantae	Tracheobionta
Division	Tracheophyta	Magnoliophyta	Magnoliophyta
Subdivision	Spermatophytina	Spermatophytina	Spermatophyte seed plants
Class	Magnoliopsida	Magnoliopsida	Magnoliopsida
Order	Fabales	Lamiales	Lamiales
Family	Fabaceae	Oleaceae	Lamiaceae
Genus	Clitoria L	Nyctanthes	Ocimum L.
Synonym	Butterfly-Pea	Night Jasmine	Holy Basil

Clitoria ternatea plant commonly known as butterfly-Pea, part of plant used for therapeutic in many issues like constipation, indigestion, nerve diseases, skin disease, arthritis, liver and intestine(10,11). It is having potential application in field of modern medicine(12). The leaves contain 3-rutinoside, 3-orhamnosyl, 3-o-rhamnosylgalactoside of kaemferol, glucoside, 3-neohesperidoside and β -sitosterol(13,14). By all the above properties it can be said that plant leaves can be used for medicinal herbal extract. *Nyctanthes arbor-tristis* plant is used in Yunani systems, Ayurvedic and Siddha-Ayurvedha medicines(15). Plant leaves possess a very wide range of bio molecules which has made a rich source of various types of medicines. They are very good properties of antipyretic, anti-viral, anti-inflammatory, antifungal, and anti-bacterial activity(16).

The plant is having some phyto chemical like flavanoids, oleanic acid, tannic acid, glycosides, essential oils, carotene, lapel, D-mannitol, nicotiflorin, glucose and benzoic acid(17,18,19).

Ocimum sanctum plant has been the part of human kind from Vedic period. The every part of plant contains medicinal value. It has many species namely *Ocimum sanctum* Linn, *Ocimum basilicum* Linn, *Ocimum canussims* many more. Among this entire species *Ocimum sanctum* has been well documented for its therapeutic potential(20). The main chemical constituents of *Ocimum sanctum* are, Linalool, Ursolic acid, Oleanolic acid, Eugenol, Rosmarinic acid, Carvacrol, and β -caryophyllene, have been used extensively for many years in food products, perfumery, and dental and oral products and plant extract continues the numerous searches for more effective drugs of plant origin in which are less toxic and available for socio-economic population in the treatment of diseases caused by pathogenic bacteria(21,22).

MATERIALS AND METHODS

Plant Leaves Collection

Traditional medicinal leaves of *Clitoria ternatea*, *Nyctanthes arbor-tristis*, and *Ocimum sanctum* are collected in month of July 2022 from Siddara Betta region, Tumkur, Karnataka, India. The leaves were authenticated, washed and sun dried for multiple days. Later they kept in hot air oven at 60°C and finely powdered with the help of mechanic grinder. The 20 gram dried powder of each leaves was

utilized for ethanol and methanol extraction. The (Figure 1) represents pictorial representation of leaves.



Figure 1: Leaves of *Clitoria ternatea*, *Nyctanthes arbortristis* and *Ocimum sanctum*

Preparation of Methanol and Ethanol extract

The dried powdered leaf samples were stored in moisture less container at room temperature. Each leaves of 20gram powder is taken i.e., totally 60 gram powder has been weighed. The powdered materials were then extracted using solvent methanol (300ml) and ethanol (300ml) separately by Soxhlet extractor. The entire process was run around 17-18h till the last drop of extract becomes colorless. The obtained extracts were concentrated in vacuum around 60°C using a rotary evaporator. The ethanol solution was evaporated using hot plate evaporator. The obtained extracts are stored in air tight container for further studies.

Culturing of Bacteria and Test for Antibacterial Activity

Active cultures of bacteria namely *Salmonella typhimurium* (gram negative), *Escherichia coli* (gram negative), *Bacillus Subtilis* (gram positive) and *Entero Bacteria* (gram negative) were purchased from MTCC, Chandigarh, India. Bacteria's are cultured under aseptic conditions in nutrient broth to result in the stock culture. The inoculated stocks of culture were incubated at 37°C for 18-24 h. The organisms were sub cultured using the stock, 24 hour prior to the experimentation. The broth was incubated at 37° C in a shaking incubator at 180 rpm. After 18-20h, nutrient agar plates were prepared by pouring the molten media on the sterile Petri plates. Onto each plate 0.1ml of the culture from the broth was transferred and spread uniformly using sterile L-shaped glass spreader. Four equidistant wells of 0.5 cm diameter were made on each plate. The experiment was repeated in duplicates of each of the organism and the results are expressed as average of the experiments.

RESULTS

Yield of the Plant Extract

The 60gram dried powder sample was extracted with 300ml of ethanol and 300ml of methanol separately. The yield of the herbal enema extract was in form of liquid, around 100ml of ethanol extract were collected in the round bottom flask and around 110ml of

methanol extract was collected after the Soxhlet extraction. After separation from methanol using rotary evaporator the volume of solution was found to be around 80ml. The boiling point of the solution was noted to be 50°C. The ethanol separation was carried out using hot plate evaporator. The collected solution was around 75ml. The boiling point of the ethanol based solution was around 50°C. Both the solutions are stored in clean glass containers and processed to check the antibacterial activity.

Antibacterial Activity of Plant Extracts

All the experiment was conducted in duplicates, and results are expressed as mean inhibition \pm Standard deviation (n=2).The tetracycline disc is used as positive control and its effect was recorded for comparison against the test samples. In the 0.5cm well made up in the petriplates are filled with concentration of extracts of 10 μ l, 20 μ l and 50 μ l solution of methanol and ethanol extract respectively. After incubation for recommended time the diameter of the zone of inhibition was recorded (Table 2). The average of the diameter of zone was considered for the final interpretation of

the effect of the plant extracts. The petriplates which were considered for experimentation is shown in (Figure 2). Duplicate of ethanol and methanol extracts were shown one after the other. (Figure 2(a)) represents the outcome of ethanol based solution whereas (Figure 2(b)) represents the methanol based solution.

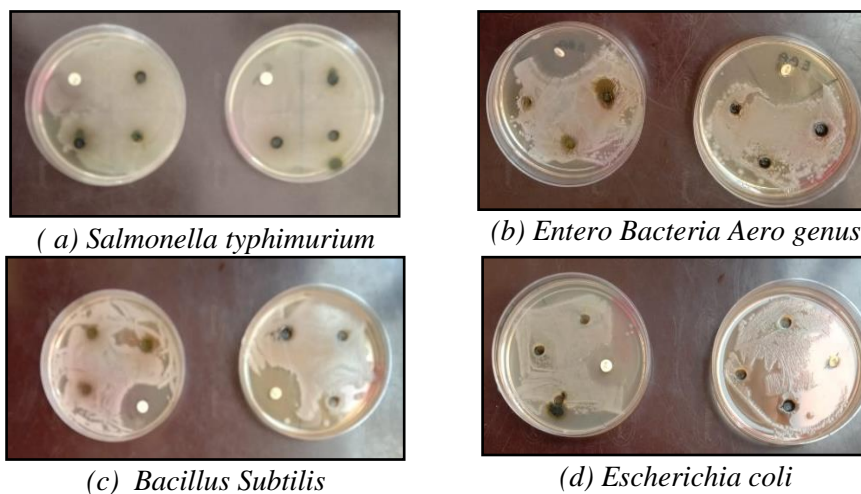


Figure 2(a): Petri plates to measure inhibition zone of Ethanol as a solvent

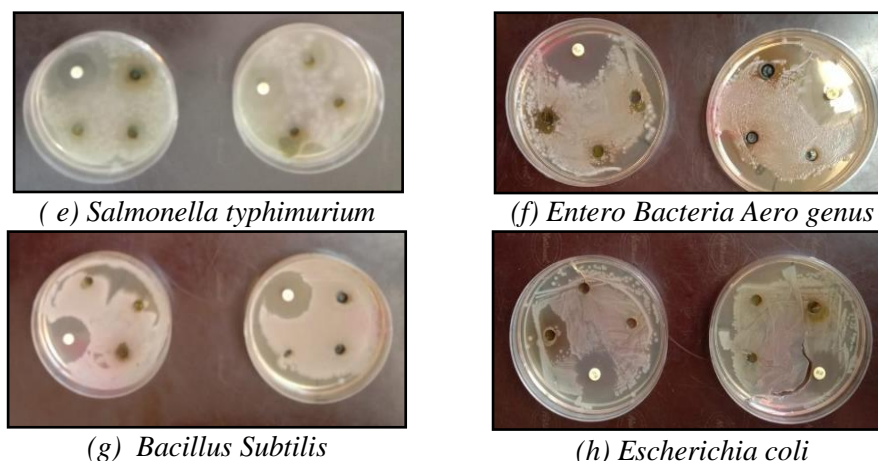


Figure 2(b): Petri plates to measure inhibition zone of Methanol as a solvent

Figure 2: Petri plates in which species are cultured and exhibited Anti bacterial activity.

Table 2: Zone of Inhibition in centimeters

Species	Standard Inhibition Zone	Ethanol Herbal Extract			Methanol Herbal Extract		
	Tetracycline	10µl	20µl	50 µl	10µl	20µl	50 µl
<i>Salmonella typhimurium</i>	2cm	0.50cm	1.00cm	2.00cm	1.00cm	1.50 cm	2.00cm
<i>Enterobacteria</i>	3cm	0.25cm	0.50cm	1.00cm	0.50cm	0.75cm	1.50cm
<i>Bacillus Subtilis</i>	2.5cm	0.25cm	0.75cm	1.25cm	0.50cm	0.75cm	1.00cm
<i>Escherichia coli</i>	2cm	1.00cm	1.25cm	1.50cm	0.25cm	0.50cm	1.25cm

The graph is plotted against by considering the bacterial species is considered on x axis and inhibition zone is considered in Y- axis (the values are average of duplicates). The (Figure 3) represents the

graphical representation of inhibition zone of bacteria indicating the effect of ethanol and methanol extracted herbal enema.

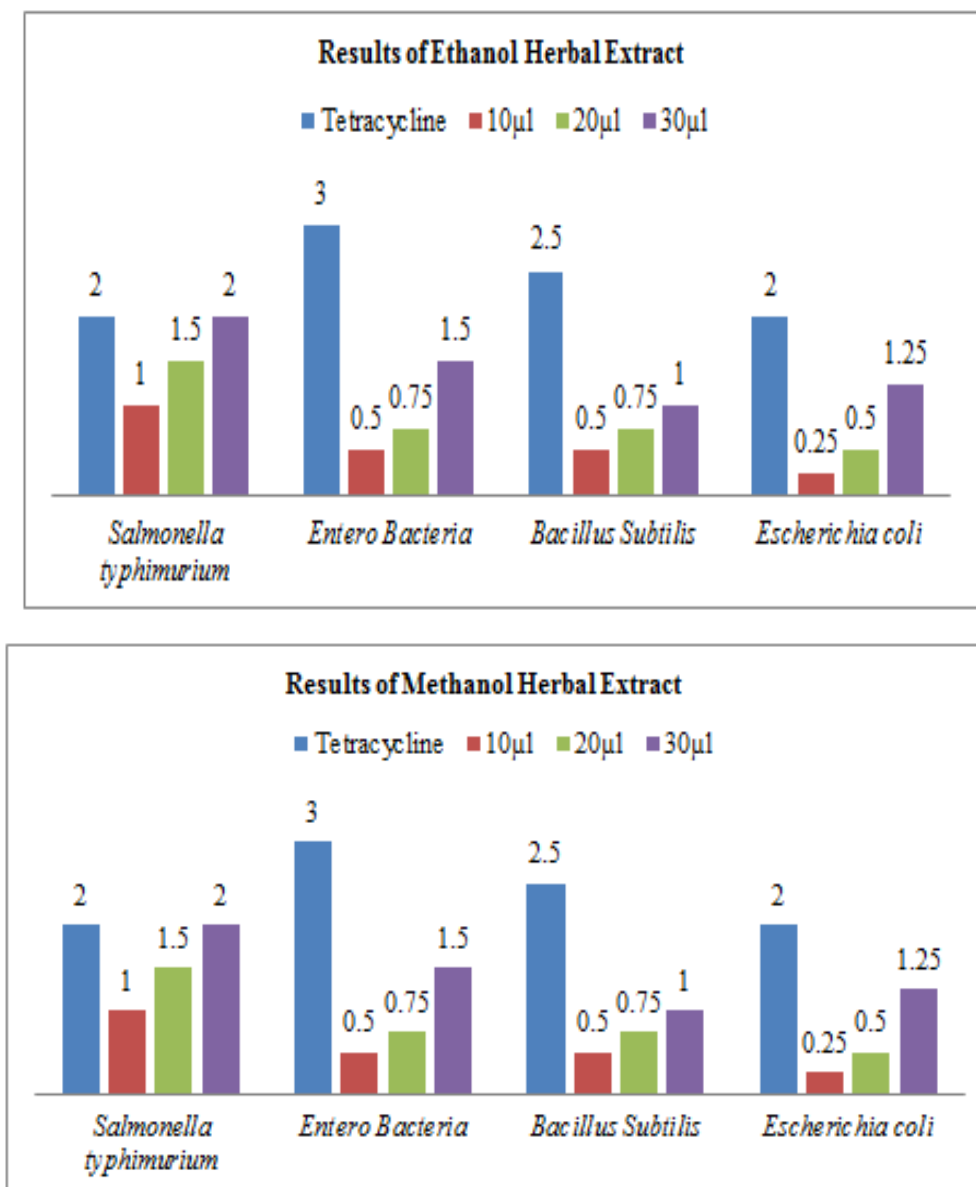


Figure 3: Graphical representation of inhibition zone of bacteria indicating the effect of Ethanol Herbal Extract and Methanol Herbal extract.

Drug Delivery Device for Herbal Enema

Liquid enema technique performed through radiological usage method as guidance is the non-operative reduction techniques. An herbal enema is used to perform the reduction procedure. It is considered as an effective technique to reduce, one that is quicker, less radiation-exposed, and safer. Repeated enema techniques can be followed with regular interval. The device is portable in which herbal enema can be targeted to pass inside anus through sucking tube. The pumping device at particular pressure can pumps the liquid. The proposed device can be further referred for clinical trials. The device is represented in (Figure 4(a)) and flow of the operation of the same is represented in the (Figure 4(b)). The surgical method is entirely removing of intussuscepted part, whereas non surgical methods avoid anesthesia and operation. The proposed technology is preferable as extracted leaves solution possess the anti bacterial solution and proposed leaves are good to cure intestinal problems.

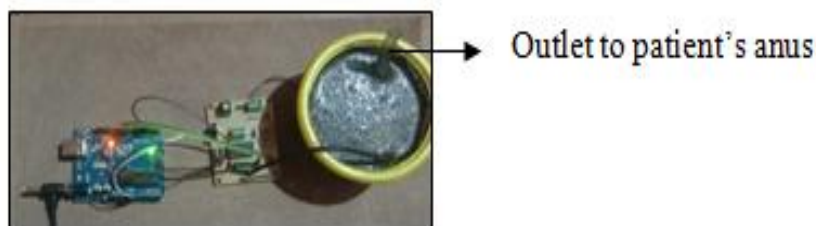


Figure 4 (a): Target Drug delivery System

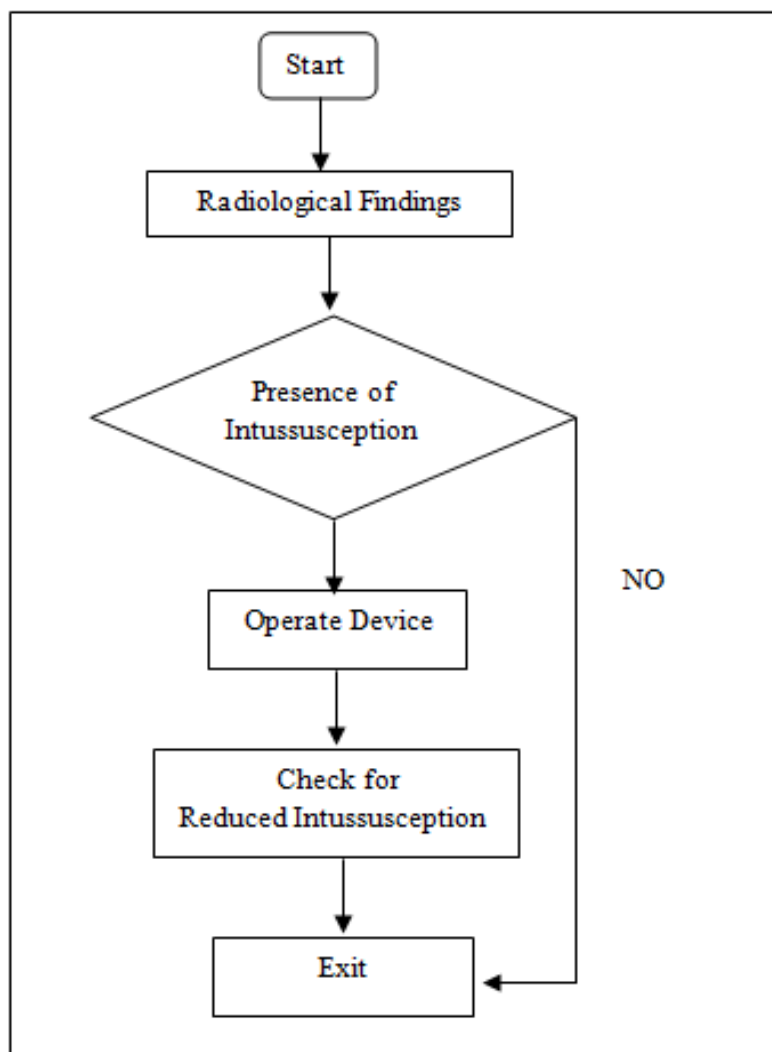


Figure 4(b): Flow Chart of Therapeutic Enema Device Operation

DISCUSSION

A new non-operative method of reduction intussusception has been proposed. The proposed herbal enema compared to pneumatic method, hydrostatic method and barium solution method, an herbal enema possesses antibacterial activity. Traditional medicinal plants are the richest bio-resources for drugs of medicines. *In-vitro* bacterial testing has been carried out by using herbal enema extracts which are obtained from leaves of *Clitoria ternatea*, *Nyctanthes arbortristis*, and *Ocimum sanctum*. They are tested against bacterial species *Salmonella typhimurium*, *Escherichia coli*, *Bacillus Subtilis* and *Enterobacteria*. It is noted that methanol and ethanol extract solution exhibits antibacterial activity. The target delivery device which has been assembled is capable of passing herbal enema in liquid form. Compared to present technology the new method is a safe method of reduction as enema is made up of herbal solution which has medicinal values. The clinical trials in the further days will lead to practical implementation of above said for curing intussusception. Herbal enema exhibits the property which reduces the bacterial activity in the intestine of the human body.

CONCLUSION

To suit the requirements for reducing intussusception an herbal liquid enema and the drug delivery system is developed. The herbal enema exhibited antibacterial activity which has been proved by *in-vitro* susceptibility test. The device has special qualities including the ability to pass herbal enema liquid as needed. The practical implementation can be achieved after ethical clearance and clinical trials. Comparatively existing methods don't possess antibacterial activity. The proposed solution is a very good antibacterial solution which can be used as enema and reduce intussusception occurrence. The electronic device can pump herbal enema which avoids human intervention where a person cannot maintain consistency speed in air or liquid outflow. The proposed method is user friendly, reliable and efficient for usage in Clinic's.

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