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Electrolyte Therapy using Herbal Extract of Ginger as Antidiarrheal Treatment

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ABSTRACT

The aim of research work to formulate ORS with ginger for treatment of diarrhea. Diarrhea causes dehydration of body fluids. Dehydration happens when your body loses more fluid than you consume. This can happen due to many reasons, including excessive diarrhea or vomiting. When left untreated, dehydration can be dangerous. Oral rehydration salts is a treatment for dehydration. It involves drinking a beverage made of water, sugar, and electrolytes, specifically potassium and sodium. The beverage is called an oral rehydration solution (ORS).The goal of oral rehydration therapy is to replenish the body's fluid levels. With the formula of ORS, the addition of Ginger plays a vital role to reduce the diarrheal effects. This research work involves the pre-formulation study followed by formulation, evaluation of product and different microbiological tests of ORS and Ginger. The culture media was prepared and microbe E.Coli added to it with ginger extract. The antimicrobial activity of sample was checked. The measurement of the optical density in the test tubes can be related to the bacterial growth, to detect the growth of the bacteria, we measured the optical density of the culture and later minimum inhibitory concentration is measured. The results showed our hypothesis that ginger inhibits the growth of Escherichia coli are therefore detected. Hence, it is confirmed that addition of ginger extract in ORS is effective treatment against diarrhea.

KEYWORDS: *ORS, Electrolyte, Ginger, E.Coli, Antimicrobial Activity*

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INTRODUCTION

Dehydration is a lack of total body water with an accompanying disruption of metabolic processes. It occurs when free water loss exceeds free water intake, usually due to exercise, disease, or high environmental temperature. Also, diarrhea is main cause of dehydration. [(1)]

Globally, diarrheal disease is a second leading cause of under-five mortality, and it contributes about 4,80,000 deaths per year. Dehydration leads to loss of water. Children age <5 years is the most suffering age group for diarrheal condition. These deaths are most entirely preventable if dehydration is prevented or treated. Until 1970, intravenous infusion of fluids and electrolytes was the treatment and choice for diarrheal dehydration but was expensive. [(2)]

Electrolyte therapy/ Oral rehydration salts (ORS) easily available in markets as a treatment of dehydration and diarrheal condition. It has more efficacy and compatibility than the IV infusion. ORS was first introduced at the end of the 1960s to replace water and electrolyte losses in diarrheal diseases. It is a combination of sugar and electrolyte solution. ORS is the simple and inexpensive; it is used to treat as a result of diarrhea and can be given at home by mother or care givers during episodes of diarrhea. Previous studies indicated that deaths from diarrheal diseases have significantly declined following the introduction of ORS. For the last two decades, evidences shows that under 5 mortality diseases has declined from 2.7 million to 0.5 million in 2015, and 4,80,000 in 2020.[(2)]

Now days it is seen that many people believe in herbal formulation as it have fewer side effects than chemical drugs. So, the main interest of the research work is to introduce herbal additives in ORS formulation. Turmeric, aloe vera, neem, sandalwood,

tulsi, amla etc. Herbal substances widely used in many disease condition, and it have great patient acceptance. Likewise Ginger (*Zingiber officinale)* has the activity, which is mainly causes diarrhea. On the basis of antibacterial activity of ginger against E-coli, the addition of ginger with ORS as a treatment of dehydration to make it more effective and reliable.[(1)]

Composition of oral rehydration salts

Oral Rehydration Salts (ORS) is the non-proprietary name for a balanced glucose-electrolyte mixture, first used in 1969 and approved, recommended, and distributed by UNICEF and WHO as a drug for the treatment of clinical dehydration throughout the world. In 1984, another mixture containing trisodium citrate instead of sodium hydrogen carbonate (sodium bicarbonate) was developed with the aim of improving the stability of ORS in hot and humid climates. For more than 20 years, WHO and UNICEF have recommended this single formulation of ORS to preventor treat dehydration from diarrhoea irrespective of the cause or age group affected. This product, which provides a solution containing 90 meq/l of sodium with a total osmolarity of 311 mosm/l, has proven effective and without apparent adverse effects in worldwide use. It has contributed substantially to the dramatic global reduction in mortality from diarrhoeal disease during the period. During this period, numerous studies have been undertaken to develop an "improved" ORS.

The goal was a product that would be at least as safe and effective as standard ORS for preventing or treating dehydration from all types of diarrhoea but which, in addition, would reduce stool output or have other important clinical benefits. One approach has consisted in reducing the osmolarity of ORS solution to avoid possible adverse effects of hyper tonicity on net fluid absorption. This was done by reducing the solution's glucose and salt (nacl) concentrations.

Studies to evaluate this approach were reviewed at a consultative technical meeting held in New York (USA) in July 2001 (4), and technical recommendations were made to WHO and UNICEF on the efficacy and safety of reduced osmolarity ORS in children with acute non-cholera diarrhoea, and in adults and children with cholera.

WHO ORS (Modified ORS)

In 1975, the WHO first introduced an ORS that subsequently has been used throughout the world for more than 25 years. This ORS was initially designed to treat children with diarrhoea from cholera. The standard WHO-ORS has an osmolarity of 311 mosm/L and concentrations of sodium at 90 meq/L, potassium at 20 meq/L, chloride at 80 meq/L and glucose at 20 g/L. However, subsequently, it became clear that the composition of the standard WHO-ORS could not be optimized to help reduce the volume of stool and duration of diarrhoea, although hydrational status could be maintained. The concentration of sodium was too high for well-nourished children with noncholera diarrhoea. This has led to a search for an ORS with improved compositions. [(5)]

Despite the great success of ORS in the treatment of acute infectious diarrhoea over the ensuing quarter century, there have been several major efforts to modify the composition of ORS with the goal to improve its efficacy (as demonstrated in clinical trials) to reduce diarrhoea (i.e., reduce both the time to first formed stool and stool volume) and its effectiveness (i.e., as established in field conditions). The use of one or more amino acids, disaccharides, and polymers (e.g., sucrose) added to ORS provided modest but not dramatic improvement in efficacy. Major efforts have been made to employ food-based, cereal-based ORS formulations. The initial ORS formulation (often referred to as WHO-ORS) is "isoosmolar" (i.e. 311 mosm/kg H_20). Since food-based formulations result in hydrolysis of oligosaccharides and peptides in the proximal small intestine, resulting in the release of substantial amounts of amino acids and hexoses, these food-based ORS formulations have, in general, been hypo-osmolar (e.g., ~245 mosm/kg H_20). Several appropriately designed randomized controlled trials have subsequently demonstrated that such formulations are significantly better than WHO-ORS (i.e., iso-osmolar). However, the question was raised whether the improved efficacy of meal-based ORS formulations was not due to the presence of food polymers per se but was a result of the hypo-osmolality of these formulations. As a consequence, a series of studies were performed with hypo-osmoles ORS formulations comparing glucose and food-based compositions. These studies established the efficacy of hypo-osmolar, glucose-based formulations (without the presence of food polymers), which represented yet another milestone in the improvement of ORS. Since then, several governments in Asia and Africa have adopted the use of reduced osmolarity (or hypo-osmolar) ORS formulation as the standard ORS treatment for diarrhoea.

Despite the ready demonstration that employment of ORS during episodes of acute diarrhoea improves morbidity and mortality especially in young children, the actual usage of ORS has varied markedly over the past 30 years for many reasons and remained relatively low and unchanged in many countries. Early on, there were extensive media events promoting employment of ORS during episodes of

acute diarrhoea. These campaigns have been judged effective to increase ORS uptake but have usually been intermittent in duration. Further, such efforts have frequently been superseded by maternal education programs that have focused on providing education addressing the totality of child welfare, with emphasis on breast feeding, vaccination programs, and other important health, nutrition, and hygiene issues for children, in addition to the employment of ORS, with consequent loss of focus on the latter. To maintain continued high levels of ORS, it is necessary to ensure continual media education. This need for continued and sustained education is critical, if only to provide sustained education of ORS for the women who become new mothers every year. Though deaths from diarrhoea are decreasing, it is important to emphasize that acute diarrhoea remains the second highest cause of mortality in children under the age of 5 in developing countries (and only slightly less than that of pneumonia).

UNICEF/WHO released an important monograph in 2009 entitled Diarrhoea: Why children are still dying and what can be done? Data presented in this publication emphasized that overall use of ORS by mothers in developing countries was only approximately 33 %. This figure is far too low and certainly may be an important factor why children are still dying from episodes of acute diarrhoea. An adequate explanation for this overall low use of ORS in the treatment of acute diarrhoea is not totally known. In addition to cultural and access issues, an important issue is that ORS is not a drug (and hence at risk of not being perceived as a medicine of real value), nor is it expensive, and therefore may be considered as not as effective as treatments that are expensive and must be purchased from pharmacies (e.g., antibiotics). Though all these reasons are distinct possibilities, we believe that a major contributing factor for ORS not being widely employed is its inability to reduce stool output dramatically. That is, mothers are most interested in relief of their child's symptoms, i.e., reduction in diarrhoea, and are not necessarily concerned about correction of acute dehydration and metabolic acidosis.[4]

Table: 1 Old ORS VS WHO (MODIFIED) ORS

(Based on conc.)

Constituents	Older	New ORS (WHO ORS)
Sodium	3.5	2.6
Glucose	20	13.5
Potassium	1.5	1.5
Citrate	2.9	2.9

Table: 2Old ORS VS WHO (Modified) ORS

(Based on osmolarity)

Constituents	Older	New ORS (WHO ORS)
Sodium	90mm	75mm
Glucose	110 mm	75 mm
Potassium	20 mm	20 mm
Chloride	80 mm	65 mm
Citrate	10 mm	10 mm
Total	311 mosm/lit	245 mosm/lit

Ginger:

Figure: 1 Ginger

Ginger, the rhizome of Zingiber officinale, is a member of the Zingiberaceae family that has been used as a spice globally. This spice contains a wide variety of volatile and non-volatile compounds with various concentrations depending on different condition of cultivation, harvesting, and processing. Chemical analysis of ginger shows that it contains over 400 different compounds. The major constituents in ginger rhizomes are carbohydrates (50–70%), lipids (3–8%), terpenes, and phenolic compounds. Terpene components of ginger include zingiberene, β-bisabolene, α-farnesene, β-sesquiphellandrene, and α-curcumene, while phenolic compounds include gingerol, paradols, and shogaol. The specific odour of ginger is related to zingiberene and bisabolene, while the pungent flavor is due to volatile oils of gingerols (23–25%) and shogaols (18–25%). Besides these components, amino acids, raw fiber, ash, protein, phytosterols, vitamins (e.g., nicotinic acid and vitamin A), and minerals are also present in ginger. Other gingerol- or shogaol-related compounds (1–10%), which have been reported in ginger rhizome, include 6-paradol, 1-dehydrogingerdione, 6- gingerdione and 10-gingerdione, 4- ginger diol, 6-gingerdiol, 8- ginger diol, and 10-gingerdiol, and diarylheptanoids. Since thousands of years ago, ginger has been used as a food and herbal medicine in Asia and the Far East so that its medical use is well described in Chinese remedies from 400 BC. The rhizomes have been used since antiquity in the various traditional systems of

medicine to treat cold, fever, sore throats, infectious diseases, arthritis, rheumatism, sprains, muscular aches, pains, cramps, hypertension, dementia, migraine, nervous diseases, gingivitis, toothache, asthma, stroke, and diabetes and also used as home remedy in treating various gastric ailments like constipation, diarrhoea, dyspepsia, belching, bloating, gastritis, epigastric discomfort, gastric ulcerations, indigestion, nausea, and vomiting. This long and established history of medicinal use of ginger in humans has stimulated clinical trials to scientifically assess the effectiveness of ginger as an adjuvant therapy or as a complementary and alternative medicine in a number of diseases especially gastrointestinal ailments. Anti-inflammatory, antioxidant, antitumor, and antiulcer effects of ginger have been proven in some studies; however, some results are controversial, probably due to the chemical instability of gingerols (the ginger most active ingredients), which are readily oxidizable substances. The U.S. Food and Drug Administration classifies ginger as "Generally Recognized as Safe" and the German Commission Monographs reported that ginger has no known side effects and no known drug/herb interactions. In this review, we summarize recent studies evaluating the effects of ginger consumption in gastrointestinal disorders. [7]

Composition of ORS with ginger:

The four above-mentioned basic ingredients for preparing an effective (clinically tested) oral rehydration solution. The four ingredients of ORS (glucose, sodium chloride, potassium chloride and trisodium citrate) in the concentrations described in this document yield an effective solution for rehydration and for the prevention of dehydration. The addition of other ingredients, such as other minerals (especially zinc) or vitamins, has not been shown to improve the solution's efficacy. If additional ingredients are included, they should be clearly described on the packet. Additional ingredients may increase the total and individual substance concentration of a solution. A clear distinction should be made between products recommended for treating/preventing dehydration caused by diarrhoea and preparations with compositions that are designed for replacing water. The theoretical advantage of flavoured and coloured ORS is greater acceptability, and consequently increased use. Because this, in turn, might lead to over-consumption, the WHO/ CDD Programme conducted a safety/efficacy study in Egypt and an accept ability study in the Philippines of flavoured and coloured ORS solutions. The results of these studies showed neither an advantage nor disadvantage for the flavoured and coloured ORS when compared to the standard ORS with regard to safety, acceptability and correct use. In practice, two or more types of flavouring are often needed, and saccharine is added to increase their effect. The ingredients used for flavouring ORS must be among those listed as "Generally Recognized as Safe" for their intended use by the US Food and Drug Administration (FDA) or by the US Flavour Extract Manufacturer's Association (FEMA). The responsibility for demonstrating the clinical efficacy, safety and chemical stability of such products remains with the manufacturer. Special attention must be given to the type of sweetener used. Some children with high purging diarrhoea may consume very large amounts of ORS solution. Because of difficulties in controlling the amount of ORS solution consumed per kg of body weight and per day, it is almost impossible to determine whether the consumed doses of colouring and/or flavouring agents are within the safe limits. Although not documented, it also seems that certain flavouring agents can cause allergies and other side effects, particularly in infants and small children. Finally, it must be noted that the flavouring of ORS may increase cost of the product by up to 20-30%, especially when the additional ingredients must be imported.

A majority of diarrhoeal cases are due to bacterial enteropathogens, diarrhoeagenic Escherichia coli being the most common cause in developing countries. The two main bacterial groups causing traveller's diarrhoea are diarrhoeagenic E. Coli, mainly enterotoxigenic and enter aggregative and invasive bacterial pathogens like Shigella, Campylobacter and Salmonella. Amongst the viral agents, rotavirus is the most common. Oral rehydration therapy (ORT) has been the key strategy for effective case management and has been instrumental in reducing diarrhea-related deaths. However, patients often express their dissatisfaction with ORT since it does not decrease the frequency of stools. Moreover, there is an increasing threat of drug resistance to antibiotics. Thus an important niche exists for development of cost-effective alternative approaches for the treatment of diarrhoea which can possibly be filled by the use of tested and well standardized medicinal plants. The virulent features of diarrhoeal organisms have been studied in great detail and the pathogenesis of infectious diarrhoea is largely well understood. However, most of the studies reporting antidiarrhoeal activity of medicinal plants overlook the pathogenesis of infectious diarrhoea and evaluate their efficacy on the basis of antimicrobial action alone. Targeting the virulence parameters as an alternative approach to define the divergent mechanism(s) of antidiarrhoeal activity of medicinal

plants, especially in the absence of antimicrobial activity, has been previously demonstrated. In the present study, the antidiarrhoeal activity of a hot aqueous extract (decoction) of dried rhizome powder of Z. Officinale has been explored using this approach to gain insights into its possible mechanism(s) of antidiarrhoeal activity. The results indicate that in the absence of antimicrobial activity, Z. Officinale would prevent bacterial colonization of the gut epithelia to control infectious diarrhoea due to pathogens such as enteropathogenic E. Coli (EPEC), enteroinvasive E. Coli (EIEC) and Shigella flexneri. It can also marginally control cholera; however, it may have no effect on giardiasis and rotaviral infections. (5)

GINGER:

- **Botanical name:** *Zingiber officinale*
- **Synonyms**: Rhizoma zingiberis, Zingibere.
- **Biological Source**: Ginger consists of the dried rhizomes of the *Zingiber officinale* Roscoe, belonging to **family:** Zingiberaceae.
- **Geographical Source**: It is mainly cultivated in West Indies, Nigeria, Jamaica, India, Japan, and Africa.
- **Cultivation**: Ginger plant is a perennial herb that grows to 1 m. It is cultivated at an altitude of 600 to 1,500 m above sea level. The herb grows well in well-drained rich, loamy soil, and in abundant rain fall.The rhizomes are socked in water overnight and the next morning they are scraped with a knife to remove the outer cork and little of parenchyma. The rhizomes are turned by the sides at regular intervals to facilitate proper drying. The coated or the unpeeled variety is prepared by dropping the rhizome for few minutes in boiling water, and then skin is removed such that the layer on the flat surface is removed but not in the grooves between the branches.
- **Characteristics**: The rhizomes are 5 to 15 cm long, 3 to 6 cm wide, and about 1.5 cm thick. The Jamaica ginger occurs as branches. It has a sympodial branching and the outer surface has buff yellow colour with longitudinally striated fibres. Small circular depressions at the portion of the buds are seen and fractured surface shows narrow bark, a well-developed endodermis, and a wide stele, with scattered small yellowish points of secretion cells and grayish points of fibrovascular bundles. The ginger has agreeable and aromatic odour and pungent and agreeable taste.
- **Chemical Constituents**: Ginger contains 1 to 2% volatile oil, 5 to 8% pungent resinous mass and starch. The volatile oil is responsible for the aromatic odour and the pungency of the drug is due to the yellowish oily body called gingerol which is odourless. Volatile oil is composed of sesquiterpene hydrocarbon like α-zingiberol; α-sesquiterpene alcohol α-bisabolene, α-farnesene, α-sesquiphellandrene. Less pungent components like gingerone and shogaol are also present. Shogal is formed by the dehydration of gingerol and is not present in fresh rhizome.
- **Uses**: Ginger is used as an antidiarrheal, antiemetic, positive inotropic, spasmolytic, aromatic stimulant, carminative, condiment, and flavouring agent. It is prescribed in dyspepsia, flatulent colic, vomiting spasms, as an adjunct to many tonic and stimulating remedies, for painful affections of the stomach, cold, cough, and asthma. Sore throat, hoarseness, and loss of voice are benefited by chewing a piece of ginger. (9)

Materials and Methods:

Pre-formulation study

Materials:

A. Apparatus: Beakers, Stirrer, Conical flask, Measuring cylinder, P^H Paper.

B. Equipment**:** Densitometer, Microscope.

Methods:

A. Colour: colour is identified by visible appearance.

B. Taste: Taste is identified with the help of tongue.

C. Particle size: By sieving method.

Standard sieves set is selected (sieve no: 10, 22, 36, 44, 65, 80, 100,120) arrange them in such manner that the coarsest remains at the top and finest at the bottom.. Weigh approximately 50g of sample, place the sample on the coarsest sieve no.10. Fix the above sieves set on hand sieve shaker and shaken for 20 minutes. Collect the Sample retained on each sieve into a paper, weigh the entiresample. Report the weights retained on each sieve in the table against corresponding sieve number.(1)

A. P^H:

Determination of pH using pH Paper

- Take a pH paper strip and place it on a white tile.
- Pour a drop of the sample on the pH paper using a clean dropper.
- Observe the colour of the pH paper.
- Now compare the colour obtained on the pH paper with the different colour shades of the standard colour pH chart and note down the pH value.
- Similarly, find the pH of the remaining samples using a fresh strip of pH paper and a separate dropper for each sample.(2)

B. Solubility:

Table3: solubility based classification of drug

Class	Parts of solvent required for dissolving one part of solute
Very soluble	<1
Freely soluble	1-10
Soluble	10-30
Sparingly soluble	30-100

By comparing above table we found solubility of ORS with ginger in water.[3]

C. Bulk density:

Pass a quantity of powder sufficient to complete the test through a sieve with apertures greater than or equal to 1.0 mm, if necessary, to break up agglomerates that may have formed during storage; this must be done gently to avoid changing the nature of the material. Into a dry graduated cylinder of 250 mL (readable to 2 mL), gently introduce, without compacting, approximately 100 g of the test sample (m) weighed with 0.1 per cent accuracy. Carefully level the powder without compacting, if necessary, and read the unsettled apparent volume (V0) to the nearest graduated unit. Calculate the bulk density in g per mL by the formula **m/V0**. Generally, replicate determinations are desirable for the determination of this property. If the powder density is too low or too high, such that the test sample has an untapped apparent volume of either more than 250 mL or less than 150 mL, it is not possible to use 100 g of powder sample. Therefore, a different amount of powder has to be selected as test sample, such that its untapped apparent volume is 150 mL to 250 mL (apparent volume greater than or equal to 60 per cent of the total volume of the cylinder); the mass of the test sample is specified in the expression of results. For test samples having an apparent volume between 50 mL and 100 mL a 100 mL cylinder readable to 1 mL can be used; the volume of the cylinder is specified in the expression of results[4]

D. Tapped density:

Proceed as described above for the determination of the bulk volume (V0). Secure the cylinder in the holder. Carry out 10, 500 and 1250 taps on the same powder sample and read the corresponding volumes V10, V500 and V1250 to the nearest graduated unit. If the difference between V500 and V1250 is less than or equal to 2 mL, V1250 is the tapped volume. If the difference between V500 and V1250 exceeds 2 mL, repeat in increments such as 1250 taps, until the difference between succeeding measurements is less than or equal to 2 mL. Fewer taps may be appropriate for some powders, when validated. Calculate the tapped density (g/mL) using the formula **m/Vf** in which Vf is the final tapped volume. Generally, replicate determinations are desirable for the determination of this property. Specify the drop height with the results. If it is not possible to use a 100 g test sample, use a reduced amount and a suitable 100 mL graduated cylinder (readable to 1 mL) weighing 130 ± 16 g and mounted on a holder weighing 240 ± 12 g. The modified test conditions are specified in the expression of the results [4].

Formulation:

Formulation of ORS:

Table 4: Ingredients of ORS [5]

Constituents	Quantiity of ORS (in gm)	Quantiity of ORS (in gm) (with Ginger)
Sodium	2.6	2.6
Glucose	13.5	13.5
Potassium	1.5	1.5
Citrate	2.9	2.9
Ginger	-	2.0

Procedure of ORS:

1. Weigh above all dry ingredients and place in mortar.
2. Grind them well with a pestle to make a uniform mixture of fine powder.
3. Pack the powder in a white paper, make a packet.
4. Put it in an envelope and label it.

Evaluation

Materials:

A. Culture: Saline suspension of E-coli., Nutrient broth cultures of E.coli.
B. Media: nutrient agar plates, Nutrient broth media.
C. Apparatus: Test tubes, flasks, pipette, petri plates, Cork burner, conical flask.
D. Equipment: Autoclave, Incubator, Water bath, Refrigerator.

Methodology:

The effect of ginger on the growth of non-pathogenic E.coli bacteria.

Principle:

Natural products are the major sources of drugs. These products have greater structural diversity than drugs or compounds from standards combinatorial chemistry. The use of medicinal plants for treatment of microbial diseases is well known and will document since ancient times. Medicinal plants synthesis many defensive compounds to protect themselves and predators. These compounds have anti-microbial activity. The ability of the plant extract to reduce or inhibit the growth of microorganisms or kill the pathogenic microorganisms is known as Anti-microbial activity of efficacy. Several plants species have been tested for anti-microbial properties but the

vast majority have not yet been adequately evaluated. Anti-microbial activity of plant extracts may be tested by agar diffusion method.

Procedure:

1. Prepare nutrient agar Petri plates for the growth of bacterial cultures
2. Spread the test cultures on the plates by spread plate method.
3. The test culture is used such as E-coli.
4. Aqueous Ginger extract (1ml of 5%) is added in the labelled wells and incubated.
5. Bacterial test culture plates are incubated at 32^0C to 35^0C for 48 hrs.
6. The sensitivity of the test organism to each of extracts is indicated by clear zone of inhibition around the well and measures the diameter of zone of inhibition. (6)

Minimum inhibitory concentration of Aqueous Ginger Extracts.

Principle:

Minimum inhibitory concentration is the minimum concentrations of anti-microbial compounds found to inhibit growth of particular test microorganism. Minimum inhibitory concentration (MIC) values are usually expressed in terms of mg/ml or units/ml. MIC determined by broth dilution method or solid dilution method.

Procedure:

Broth dilution method:

Prepare nutrient broth test tubes and label as shown in table. In first test tube UT (uninoculated), inoculums is not added which is used for checking the sterility of media and as a negative control. Other all test tubes, inoculums 3-4 drops is added to reach the final concentration of microorganism is 10^6 per ml. In all test tubes test anti-microbial compound is added ranging from 0.5 – 5 ml except uninoculated (negative control) and control (positive) tube. The positive control tube is used to check the suitability of the medium for growth of the test microorganism and the viability of the inoculums. Adjust the final volume (10 ml) in all test tubes by using sterile water .all test tube are properly shaken and then incubated at 37^0C for two days.(6)

Result and Discussion:

Pre-formulation studies

- **Physical characteristics**
- **Bulk Characterization**

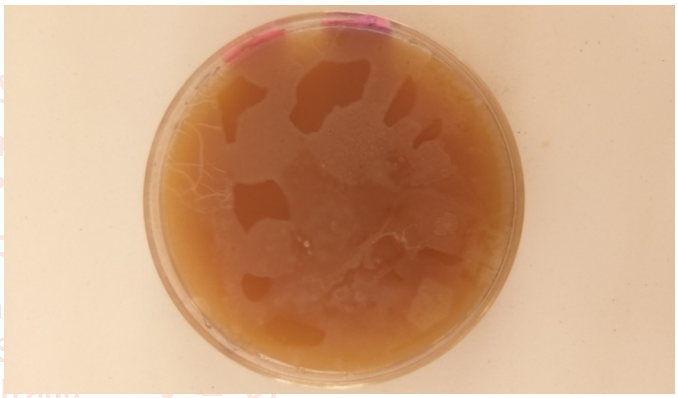
A. **Color**: White crystalline powder
B. **Taste**: strong salty and pungent taste
C. **Odour**: pungent odour
D. **Particlesize**: 1.0 mm- 1.5 mm

Stability Analysis

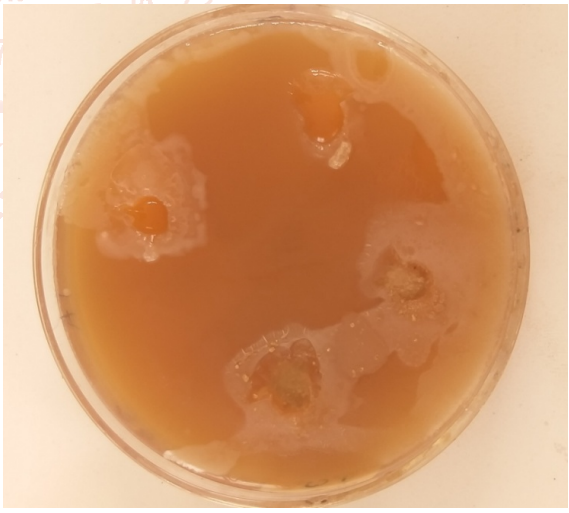
A. **P^H of ORS solution:** 7.0- 7.5
B. **Solubility of ginger with ORS:** ORS is freely soluble in water but with addition of ginger it becomes sparingly soluble in water.
C. **Bulk density:** 800 gm perliter.
D. **Tapped density:** 860 gm per liter.
E. **Compatibility:** ORS is compatible with Ginger extract.
F. **Crystallinity and polymorphism:** Crystalline in nature.
G. **Angle of repose:** 20-30 i.e., good flow properties**.**

Antimicrobial activity

1. Based on observation, the well of petri plate with added aqueous extract of Ginger, showing less growth as compared to another well having only culture media.



(Figure 2: Agar Petri plate with culture media)



(Figure 3: Agar Petri plate with culture media and aqueous Ginger extract)

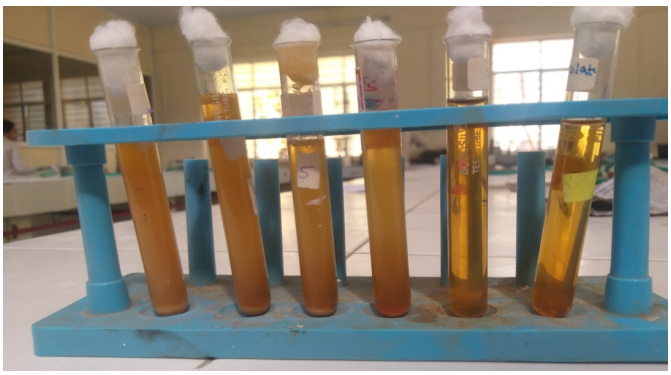
Microbial inhibitory concentration

After incubation, the some test tube showing growth and some test tube not showing growth.

The UT test tube not showing any growth, hence sterility confirms.

The CT tube showing growth hence E.coli bacteria responding to the media.

Remaining test tube out of which first five show growths and remaining five test tube do not show any growth. Hence minimum inhibitory concentration of aqueous extract of ginger having 3 ml of 5% concentration.



(Figure 4: Test tube showing MIC)

Summary:

As ORS is very important medical treatment in case severe clinical dehydration. ORS is simple, proven intervention that can be used at the community and facility to prevent and treat diarrheal dehydration and decrease diarrheal mortality. Operation and implementation research is needed to better understand how to deliever ORS effectively and promote its used at home and facility level as part appropriate case management of diarrhea. Ginger extract play a very important role in dehydration and diarrhea as it inhibits the growth E.coli microbes. E.coli is responsible bacteria for diarrhea.

On the basis of experiment and testing it is found that ORS with addition of extract of ginger effective in control the growth of E.coli hence, help to reduce the dehydration in case of diarrhea. E.coli bacteria were placed in solid and liquid growth culture media. After they had reached their exponential phase extract of ginger was added to culture media. The growth of bacteria didn't seem to be affected much in regard to the control bacteria without ginger. Experiment regarding to determination of antimicrobial activity of ginger and minimum inhibitory concentration of ginger showed us that ginger has visible effect on E.coli in 5gm in 100ml of concentration.

Conclusion:

ORS is effective against diarrhoea mortality in home, community and facility settings; however, there is insufficient evidence to estimate the effectiveness of Recommended Homemade fluids (RHF) against diarrhea mortality. Most child deaths occur due to a small number of conditions that are preventable, even in the poorest settings, through interventions that are well known, affordable and deliverable via simple technologies. The systematic review returned studies looking at a variety of interventions to increase the use of ORS to treat diarrhea in children. While the interventions in this review show promise, firm conclusions cannot be drawn due to issues with the small volume of the evidence and high levels of heterogeneity within the meta-analyses. Research is needed specifically investigating strategies to scale-up the use of ORS, looking at the system from multiple vantage points, in a range of settings where ORS use has been historically low.

With regard to its effects on the gastrointestinal tract, there have been several reports that have put forward different mechanisms of action to support the traditional use of Ginger. Constituents of Ginger such as 6-, 8-, 10-gingerols and 6-shogaol when fed orally accelerate gastric emptying, through their anti-serotonergic activity whereas intravenous injection of the same inhibited gastric motility. However, there are few studies that describe its mechanism of action in infectious diarrhea. it is our belief that the whole extract rather than the isolated fraction may prove beneficial due to the possible synergy of more than one compound. Additionally, a crude extract represents the nearest form of a traditional preparation and it is possible that pure compound(s) may not necessarily behave in the same manner as the natural extract. The inclusion of different test strains could have also contributed to the discrepancy in the results of the antibacterial activity. However, in the present study, in the absence of antimicrobial activity, Ginger seems to target other parameters to exhibit its antidiarrheal action. This implies that Ginger affects bacterial metabolism to suppress the toxin production. Though not a marker of efficacy, With regard to its effects on the gastrointestinal tract, there have been several reports that have put forward different mechanisms of action to support the traditional use of Ginger. Most of these have used animal models using physiological parameters and have proposed that constituents of Ginger counteract delay in gastric emptying, affect intestinal motility, possess gastric prokinetic activity and inhibit intestinal fluid accumulation. Constituents of Ginger such as 6-, 8-, 10-gingerols and 6-shogaol when fed orally accelerate gastric emptying through their anti-serotonergic activity whereas intravenous injection of the same inhibited gastric motility. However, there are few studies that describe its mechanism of action in infectious diarrhea. Hence in the present study, hot aqueous extract of Ginger was studied for its antimicrobial action along with its effect on bacterial virulence features.

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