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Formulation and Development of Herbal Oral Rehydration Solution (HORS)

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ABSTRACT

In physiology, 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. Diarrhea, also spelled diarrhea or diarrhea, is the condition of having at least three loose, liquid, or watery bowel movements each day. Oral Rehydration Therapy (ORT) is the cheap, simple and effective way to treat dehydration caused by diarrhoea. When diarrhoea occurs, essential fluids and salts are lost from the body and must be quickly replaced. Many countries now have diarrhoeal disease control programmes, but Oral Rehydration Therapy is still not nearly as widely used as it should be and more effective information dissemination and promotion of Oral Rehydration Therapy is necessary. A brilliant alternative will be herbal ORS (HORS) to tackle the situation effectively. In this study, HORS comprising of extracts of *Ficus religiosa* (Peepal) and *Azadirachta indica* (Neem) were formulated through granulation. Further, the anti-diarrheal activity was explored in mice.

1. INTRODUCTION

In physiology, 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 [1]. Mild dehydration can also be caused by immersion diuresis, which may increase risk of decompression sickness in divers. Most people can tolerate a 3-4% decrease in total body water without difficulty or adverse health effects. A 5-8% decrease can cause fatigue and dizziness [2]. Loss of over 10% of total body water can cause physical and mental deterioration, accompanied by severe thirst. Death occurs at a loss of between 15-25% of the body water [3]. Mild dehydration is characterized by thirst and general discomfort and is usually resolved with oral rehydration. Dehydration can cause hypernatremia (high levels of sodium ions in the blood) and is distinct from hypovolemia (loss of blood volume, particularly blood plasma) [4]. Risk factors for dehydration include but are not limited to: exerting oneself in hot and humid weather, habitation at high altitudes, endurance athletics, elderly adults, infants, children and people living with chronic illnesses [5]. Dehydration can also come as a side effect from many different types of drugs and medications. In the elderly, blunted response to thirst or inadequate ability to access free water in the face of excess free water losses (especially hyperglycemia related) seem to be the main causes of dehydration [6]. Excess free water or hypotonic water can leave the body in two ways – sensible loss such as osmotic diuresis, sweating, vomiting and diarrhea, and insensible water loss, occurring mainly through the skin and respiratory tract. In humans, dehydration can be caused by a wide range of diseases and states that impair water homeostasis in the body. These occur primarily through either impaired thirst/water access or sodium excess [7].

Diarrhea, also spelled diarrhoea, is the condition of having at least three loose, liquid, or watery bowel movements each day. It often lasts for a few days and can result in dehydration due to fluid loss. Signs of dehydration often begin with loss of the normal stretchiness of the skin and irritable behavior [8]. This can progress to decreased urination, loss of skin color, a fast heart rate, and a decrease in responsiveness as it becomes more severe. Loose but non-watery stools in babies who are exclusively breastfed, however, are normal [9]. The most common cause is an infection of the intestines due to a virus, bacterium, or parasite—a condition also known as gastroenteritis. These infections are often acquired from food or water that has been contaminated by feces, or directly from another person who is infected [10]. The three types of diarrhea are: short-duration watery diarrhea, short-duration bloody

diarrhea, and persistent diarrhea (lasting more than two weeks, which can be either watery or bloody). The short duration watery diarrhea may be due to cholera, although this is rare in the developed world [11]. If blood is present, it is also known as dysentery. A number of non-infectious causes can result in diarrhea. These include lactose intolerance, irritable bowel syndrome, non-celiac gluten sensitivity, celiac disease, inflammatory bowel disease such as ulcerative colitis, hyperthyroidism, bile acid diarrhea, and a number of medications. In most cases, stool cultures to confirm the exact cause are not required [12].

Oral rehydration therapy (ORT) is a type of fluid replacement used to prevent and treat dehydration, especially due to diarrhea. It involves drinking water with modest amounts of sugar and salts, specifically sodium and potassium [13]. Oral rehydration therapy can also be given by a nasogastric tube. Therapy should routinely include the use of zinc supplements. Use of oral rehydration therapy has been estimated to decrease the risk of death from diarrhea by up to 93% [14]. Side effects may include vomiting, high blood sodium, or high blood potassium. If vomiting occurs, it is recommended that use be paused for 10 minutes and then gradually restarted. The recommended formulation includes sodium chloride, sodium citrate, potassium chloride, and glucose [15]. Glucose may be replaced by sucrose and sodium citrate may be replaced by sodium bicarbonate, if not available. It works as glucose increases the uptake of sodium and thus water by the intestines. A number of other formulations are also available including versions that can be made at home. However, the use of homemade solutions has not been well studied [16].

Although, ORT is the cheap, simple and effective way to treat dehydration caused by diarrhea, but when diarrhea occurs, essential fluids and salts are lost from the body and must be quickly replaced. Many countries now have diarrheal disease control programmes, but ORT is still not nearly as widely used as it should be and more effective information dissemination and promotion of ORT is necessary. A brilliant alternative will be herbal ORS (HORS) to tackle the situation effectively. In this study, HORS comprising of extracts of *Ficus religiosa* (Peepal) and *Azadirachta indica* (Neem) were formulated through granulation. Further, the anti-diarrheal activity was explored in mice.

2. MATERIALS AND METHODS

2.1. Instrumentation

Spectroscopic analysis was carried out using double-beam Shimadzu® Ultraviolet-Visible Spectrophotometer (Model UV-1800, Kyoto, Japan) connected to a computer having a spectral bandwidth of 1 nm and wavelength accuracy of ±0.3 nm with a pair of 10 mm path length matched quartz cells was used. All weighing were performed using Shimadzu® electronic balance (Model AUW220D, Kyoto, Japan). Sonication was performed using Transonic Digital S (Sonicator), USA. Microscopy was per-formed using trinocular microscope CosLab® HL-24(B) equipped with Scope Image 9.0 software for recording.

2.2. Chemicals

All reagents, consumables, and chemicals for evaluation were purchased from Sigma-Aldrich (Germany) and HiMedia (India) through a local vendor at Bilaspur. Double distilled water apparatus (Borosil®, India) was used for the experiment.

2.3. Animals

Albino rats of age 5 to 6 weeks age, 150-230 g body weight were utilized. After obtaining permission from the Department Ethical Committee and with compliance with the CPCSEA, the experiment was performed on the rats kept in the animal house under the conditions of 25–26ºC temperature, humidity 50–65%, 12 hr light and dark. The rats were kept in polypropylene cages (two animals per cage), standard rodent pellets were fed, and given free access to water.

2.4. Acute toxicity studies

Accordance to the OECD protocol, the *in vivo* safety limit of the extract was estimated in the increasing dose in the range 25 mg/kg to 500 mg/kg. The safe dose of the molecules was computed based on the LD_{50} values to determine the point at which maximum therapeutic effect can be achieved without any possible toxic symptoms [17].

2.5. Collection and Authentication of plant material

The leaf of plant were collected from the tree present at the medicinal plant garden of School of Pharmacy, Chouksey Engineering College situated in Lalkhadan area of Bilaspur city in

the Chhattisgarh state of India. The plant was authenticated (No. Bot/GGV/2022/28) by Dr. A. K. Dixit, Department of Botany, Guru Ghasidas University, Bilaspur, Chhattisgarh.

2.6. Preparation of extract

The leaves were collected from the tree, dried in the shade for a specified period, and powdered suitably. The dried powder (100 g, divided into multiple smaller amounts) was subjected to continuous hot Soxhlet extraction with 50 mL distilled water and 50 mL alcohol (ethanol 90%) in equal ratio at a temperature of 55-65°C during 32 cycles. The solvent was removed under reduced pressure and controlled temperature using a rotary vacuum evaporator. The yield was found to be 11.8% w/w [18].

2.7. Phytochemical Screening

Phytochemical screening was executed for the presence of sugars, alkaloids, glycosides, tannins, flavonoids, steroids, proteins, and terpenes as per the standard test procedures [19].

2.7.1. *Alkaloids*

Hager's test: Saturated solution of picric acid was added to extract (10 mg/mL), the formation of yellow precipitate indicates the presence of alkaloids.

2.7.2. *Flavonoids*

Shinoda's test: Few magnesium turnings and concentrated HCl was added drop wise to extract (10 mg/mL), appearance of a pink scarlet or crimson red color after few minutes confirmed the presence of flavonoids.

2.7.3. *Carbohydrate*

Fehling's test: 2 mL of extract was mixed with equal volumes of Fehling A and Fehling B in different tubes and boiled for few minutes. Both the contents were mixed as they attain nearly the boiling point. The appearance of brownish-red precipitate formation indicated the presence of carbohydrates.

2.7.4. *Cardiac glycoside*

Legal's test: To extract (10 mg/mL), pyridine and alkaline sodium nitroprusside solution were added. An appearance of blood red color signified the presence of cardiac glycoside, but no blood red color appeared reflecting complete absence of cardiac glycoside.

2.7.5. *Anthraquinone glycoside*

Borntrager's test: The extract (10 mg/mL) was boiled with 1 mL of sulfuric acid in a test tube for 5 minutes and filtered while hot. The filtrate was cooled and shaken with an equal volume of dichloromethane. The lower layer of dichloromethane was separated and shaken with the half of its volume of dilute ammonia. A rose pink to red color was produced in the ammonia layer and indicated the presence of anthraquinone glycoside.

2.7.6. *Tannin*

Gelatin test: To the extract (10 mg/mL), 1% gelatin solution containing 10% NaCl was added, formation of buff-colored precipitate resulted due to the presence of tannins.

2.7.7. *Saponin*

Froth formation test: 2 mL of extract was taken in a test tube and shaken until a stable froth or foam was formed for 5 minutes (in presence of saponin), however, no foam was formed for 5 minutes indicating the absence of saponin in extract.

2.7.8. *Steroid*

Libermann-Burchard's test: The extract (10 mg/mL) was treated with 7-8 drops of acetic anhydride solution, boiled, and cooled further. Concentrated sulfuric acid (5-6 drops) was further added from the side of the test tube, where a brown ring was formed at the junction of both layers; and upper layer changed to green, which demonstrated the presence of steroids.

2.7.9. *Protein*

Xanthoproteic test: To the extract (10 mg/mL), 1 mL of concentrated nitric acid was added and boiled to get a yellow precipitate, which after cooling, were added 2 mL of 40% sodium hydroxide solution, orange color appears (if protein is present). No orange color was formed with extract indicating absence of protein.

2.7.10. *Phenol*

Ferric trichloride: The extract (10 mg/mL) was dissolved in water, and 8-10 drops of dilute ferric trichloride were added, the formation of bluish-black color indicated the presence of phenol.

2.7.11. *Diterpene*

Copper acetate test: The extract (10 mg/mL) was treated with 3-4 drops of copper acetate solution, emerald green color appeared (in presence of diterpene). In extract, no emerald green color appeared, which confirmed the absence of diterpenes.

2.7.12. *Triterpene*

Salkowski's test: The extract (10 mg/mL) was treated with 5-6 drops of concentrated sulfuric acid, yellow color formation occurs in the lower layer (if triterpene is present), however no yellow color was formed describing absence of triterpene.

2.8. Formulation development

2.8.1. Spray drying process

The spray-dried extracts (SDE) were produced on a semi-industrial scale based on the standard methodology. The solution extract was obtained by vegetable drug decoction of aerial parts, using water as an extractive solvent at 100°C for 15 minutes of extraction. The spray-dried extracts were produced in a spray-dryer (Production Minor with rotary disc atomizer) using 30% colloidal silicon dioxide (Aerosil® 200) as a drying agent based on the dry residue of the extracted solution. The pharmaceutical excipients microcrystalline cellulose – MCC (Avicel pH 101, FMC corp.), magnesium stearate, colloidal silicon dioxide (Aerosil® 200, Degussa) and Eudragit® E100 (RÖHM) were used as inert agents [20, 21].

Formulation of granules:

Ingredients	**F1**	**F2**	**F3**
Azadirachta indica	15	20	25
Ficus religiosa	25	20	15
HPMC	15	15	15
Microcrystalline cellulose	34	34	34
Aerosil	3	3	3
Magnesium stearate	3	3	3
Eudragit E100	5	5	5

2.8.2. Dry granulation

The F1 formulation was prepared by blending SDE with filler/ binder (MCC) for 15 min with addition of lubricant (ST) and glidant (CSD) and blending for a further 5 minutes. For the F2, F3 and F4 formulations the SDE was blended with excipients for 5 minutes. Each formulation was compacted by direct compression using an eccentric compression machine (Korsch EK-0) equipped with flat-faced 15 mm punch. Granulation was performed in a dry granulator (Erweka type TGIIS) Granules were screened to obtain a granulometry range of 1.0 mm to 0.25 mm. Granules below 0.25 mm were re-compacted following the same methodology as described above. The compression and screening cycles were repeated until less than 10% of final particle was obtained. The granule yield was calculated in the first granulation cycle considering the percentage of screened granules in the total mass of the formulation [22, 23].

2.9. Characterization of granules

2.9.1. Bulk density

A quantity of 2 g of powder from each formula, previously lightly shaken to break any agglomerates formed, was introduced into a 10 ml measuring cylinder. After the initial volume was observed, the cylinder was allowed to fall under its weight onto a hard surface from the height of 2.5 cm at 2-second intervals. The tapping was continued was until no further change in volume was noted [24].

LBD = Weight of the powder / Volume of the Packing

2.9.2. Tapped density

A quantity of 2 g of powder from each formula, previously lightly shaken to break any agglomerates formed, was introduced into a 10 ml measuring cylinder. After the initial volume was observed, the cylinder was allowed to fall under its weight onto a hard surface from the height of 2.5 cm at 2-second intervals. The tapping was continued was until no further change in volume was noted [25].

TBD = Weight of the powder / Tapped volume of the packing

2.9.3. Hausner's ratio

Hausner's ratio can be determined by the following equation [26],

Hausner's ratio = Tapped bulk density / Loose bulk density

2.9.4. Angle of repose

Its significant parts of the tablet definitions are the point of rest of granules was controlled by the funnel technique. The precisely gauged granules were taken in a funnel. The stature of the channel was balanced so that the tip of the funnel contacts the zenith of the pile of the granules. The granules were allowed to flow through funnel freely onto the surface [27]. The diameter of the powder cone was measured, and the angle of repose was calculated using the following equation:

$$q = \tan^{-1} h/r$$

Where, q = angle of repose, h = height of the cone, r = radius of the cone base

2.9.5. Carr's Index

Carr's Index relates the poured density of the material to the tapped density and was calculated by using the following relationship [28]:

Carr's Index = Tapped density − Poured density / Tapped density × 100

2.10. Anti-diarrheal activity

The assimilation of methods was followed for this study. Only animals which were found diarrheic when they have taken 0.5 ml castor oil in the initial screening test were included in this experiment. Mice fasted for 24 h were randomly allocated to five groups of six animals each. For the induction of diarrhea 0.5 ml castor oil was given for each mouse 30 min before treatment. Each animal was then placed in individual cage, the floor of which was lined with transparent paper and every hour the floor lining was changed. Onsets of diarrhea, total number of fecal outputs within in the 4 h period were recorded. And even total fluid content of the faces was determined by using the weight difference of the fresh and dry stool (dried for 24 h at room temperature in a shaded area). Evacuation classification based on stool consistency was assigned as follows: normal stool = 1, semi-solid stool = 2 and watery stool = 3 and mean of evacuation index (EI) was calculated for each group. For all the groups the percentage inhibition of diarrhea was also calculated compared to the negative controls [29]. In all models the mice were randomly grouped (n = 6) and the substances administered were as follows:

- Group I: received 1 ml/100 mg normal saline (NS).
- Group II: treated with standard drug, 3 mg/kg lopiramide.
- Group III: treated with 100 mg/kg extract of *Azadirchta indica*.
- Group IV: treated with 100 mg/kg extract of *Ficus religiosa*.
- Group V: treated with Herbal ORS (HORS) Formulation F3 (100 mg/kg dose).

2.11. Antimicrobial activity

Modified agar well diffusion method was used to detect the antibacterial activities of different extracts and formulations. In this method, each nutrient agar plates were planted with 0.2mL of 24 hr broth culture of *Escherichetia coli.* The plates were dried for 1 hr. in each of the plates, equidistant wells were excavated with a sterile 8mm borer. Into each plate, 0.5 mL of solutions of extract and clindamycin (standard drug) were introduced. The plates of were incubated at 37^0C $\pm 1^0$C for 24 hr. The diameter of the zones of inhibition (in mm) was measured for evaluating the antibacterial activity. The experiment was repeated three times and the mean was recorded. Dimethyl sulfoxide(DMSO) was employed as negative control.

2.12. Statistical analysis

All the experimental data are expressed as mean ± Standard error of means. For data processing and analysis SPSS statistical software v.21.0 was used. Statistical significance of differences between groups was assessed by One-way ANOVA followed by post-hoc Tukey's multiple Comparison Test. The results were considered significant at p-value less than 0.05.

3. RESULTS AND DISCUSSION

3.1. Extracts

3.1.1. Percentage Yield

The extractive yield was found to be 11.63% for *Azadirachta indica* and 9.39% for *Ficus religiosa*.

3.1.2. Phytochemical analysis

Azadirachta indica showed the presence of carbohydrate, saponin, alkaloid, tannin, flavonoid, and terpenes **(Table 1)**.

Table 1. Phytochemical analysis of *Azadirachta indica*.

Chemical constituent	**Test performed**	**Observations**	**Inference**
Alkaloid	Hager's test	Yellow precipitate	**Alkaloid present**
Flavonoid	Shinoda's test	Pinkish-red color	**Flavonoid present**
Tannin	Gelatin test	Green color appeared	**Tannin present**
Glycoside	Borntrager's test	No Faint pink color observed	Anthraquinone glycoside absent
Cardiac Glycoside	Legal's test	No red color observed	Cardiac glycoside absent
Saponin	Froth formation test	A small height froth formed for 5 min	**Saponin present**
Carbohydrate	Fehling's test	Red precipitate	**Carbohydrate present**
Phenol	$FeCl_3$ test	Bluish-black color observed	Phenol present
Protein	Xanthoprotic test	No yellow color observed	Protein absent
Sterol	Libermann-Burchard's test	No Brown-ring formation	Sterol absent
Diterpene	Copper acetate test	Emerald green color observed	**Diterpene present**
Triterpene	Salkowski's test	Yellow color observed	**Triterpene present**

Ficus religiosa showed the presence of carbohydrate, saponin, alkaloid, tannin, flavonoid, and terpenes **(Table 2)**.

Table 2. Phytochemical analysis of *Ficus religiosa*.

Chemical constituent	**Test performed**	**Observations**	**Inference**
Alkaloid	Hager's test	Yellow precipitate	**Alkaloid present**
Flavonoid	Shinoda's test	Pinkish-red color	**Flavonoid present**
Tannin	Gelatin test	Green color appeared	**Tannin present**
Glycoside	Borntrager's test	No Faint pink color observed	Anthraquinone glycoside absent
Glycoside	Legal's test	No red color observed	Cardiac glycoside absent
Saponin	Froth formation test	A small height froth formed for 5 min	**Saponin present**
Carbohydrate	Fehling's test	No Red precipitate	Carbohydrate absent
Phenol	$FeCl_3$ test	Bluish-black color observed	Phenol present
Protein	Xanthoprotic test	No yellow color observed	Protein absent
Sterol	Libermann-Burchard's test	Brown-ring formation	Sterol present
Diterpene	Copper acetate test	Eemerald green color observed	**Diterpene present**
Triterpene	Salkowski's test	Yellow color observed	**Triterpene present**

3.2. Characterization of granules

The evaluated granules were observed to be acceptable as per the limits. The densities (both bulk and tapped) lie in the range of 0.742-0.769 g/cm^3 and 0.838-0.862 g/cm^3, respectively, which represented an excellent packing of the granules. The angle of repose was observed to be 28.02°-30.08° for the batches which indicated reasonably satisfactory flow property. The calculated Hausner's ratio and Carr's index was in the order of 8.87-12.42% and 1.10-1.14, respectively which may be interpreted as relatively good packing ability. In short, the tablet blend demonstrated fairly good micromeritic attributes essential for exhibiting sustained release characteristics. The pre-compression parameters are shown in **Table 3**.

Table 3. Characterization of granules.

Formulation	Angle of repose (°)	Bulk density (in g/mL)	Tapped density (in g/mL)	Carr's index (in %)	Hausner ratio
F1	29.17±0.52	0.742±0.013	0.848±0.00745	12.42±1.94	1.14±0.025
F2	30.08±0.53	0.769±0.0098	0.862±0.0022	11.7±1.24	1.13±0.017
F3	28.02±0.48	0.763±0.013	0.838±0.0065	8.87±1.75	1.10±0.019

3.3. Biology

3.3.1. Acute toxicity study

Accordance to the OECD protocol, the *in vivo* safety limit of the extract was estimated in the increasing dose in the range 25 mg/kg to 500 mg/kg where no signs or symptoms of toxicity, redness, fatal signs, etc. were observed.

3.3.2. Anti-diarrheal activity

Considering the latency of defecation after castor oil supplementation, only the 400 mg/kg extract dose treated groups demonstrated significant ($p < 0.01$) delay compared to the negative controls. The onset of defecation in this group was also found significantly ($p < 0.05$) different compared to the 100 mg/kg extract dose treated groups. Like that of the standard drug, the hydroethanolic crude extract showed statistically significant ($p < 0.05$) inhibition both in the frequency of defecation and total weight of the fluid content of the faces compared to the negative controls. The percentage inhibition of diarrhea by the 100 mg/kg doses of the *Azadirachta indica*, *Ficus religiosa*, and Herbal ORS (HORS) Formulation were determined 22.49, 43.62, and 53.51 %, respectively **(Table 4)**. And this inhibition, especially from the largest dose of extract was comparable with the inhibitory effect (51.02%) of lopiramide. Neither the positive control nor the extract treated groups exhibited statistically significant difference in the mean evacuation index compared to the normal saline exposed groups. The mean evacuation index didn't show apparent difference among any of the groups.

Table 4. Anti-diarrheal activity.

Group	Onset of diarrhea (min)	Total number of faeces in 4 h (frequency of defecation in 4 h)	Mean evacuation index	Fluid content of the faces (g)	% Inhibition of diarrhea
NS	63.33 ± 5.58	11.83 ± 1.30	2.39 ± 0.09	0.78 ± 0.13	0.00
3 mg/kg Lop	99.17 ± 18.14	5.83 ± 0.75*	2.29 ± 0.15	0.36 ± 0.05*	51.02
100 mg/kg *Azadirachta indica*	71.83 ± 5.67	9.17 ± 0.70	2.34 ± 0.06	0.61 ± 0.11	22.49
100 mg/kg *Ficus religiosa*	75.33 ± 4.86	6.67 ± 1.52*	2.17 ± 0.08	0.32 ± 0.09*	43.62
100 mg/kg Herbal ORS (HORS) Formulation	121.17 ± 12.18*[ab]	5.50 ± 0.67*	1.96 ± 0.16	0.35 ± 0.08*	53.51

3.3.3 Antimicrobial activity

Ficus religiosa extract and *Azadirachta indica* extract individually showed low antibacterial activity whereas both *Ficus religiosa* extract and *Azadirachta indica* extract containing HORS formulation expressed moderate antibacterial activity with average MIC value against E. coli **(Table 5).**

Table 5. Antimicrobial activity of HORS

Components	E. coli
Ficus religiosa extract	13.6 ± 1.17*** (12.5)
Azadirachta indica extract	16.9 ± 1.43*** (12.5)
Ficus religiosa +*Azadirachta indica* extract (HORS formulation)	19.3 ± 1.33*** (12.5)
Clindamycin #	29.9 ± 1.57(6.25)

All values represent mean ± SEM of n= 3***p<0.001. zone of inhibition of test compounds against microbes are measured in mm. values inside the bracket represents the minimum inbitory concentration(MIC). #standard reference for antibacterial activity.

4. CONCLUSION

In conclusion this study verified that other than being safe up to a dose of 2000 mg/kg, hydroethanolic crude extracts have antidiarrrheal activity. Accordingly, the study validates traditional use of the plant in form of herbal ORS (HORS) for diarrhea and may guide us to use it as a potential source of new agent in the therapeutic armamentarium of diarrhea.

Conflict of interest

No conflict of interest is declared.

Funding information

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