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Research Article

Formulation and evaluation of *in vitro* antacid effect of effervescent granules containing extracts of *Evolvulus alsinoides*

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ABSTRACT

Purpose: This study investigated the *in vitro* antacid activity of aqueous and ethanolic extracts of *Evolvulus alsinoides* Linn. plant and formulated an antacid dosage form subsequently.

Method: Extracts were prepared by refluxing whole plant of *E. alsinoides*. The activity of the extracts was evaluated by *in vitro* neutralising of artificial gastric acid where the neutralising effect as well as the neutralising capacity were determined. The freeze-dried powder of the aqueous extract of *E. alsinoides* was used to formulate effervescent granules. Antacid effect of the effervescent granules was evaluated using Vatier's artificial stomach model in addition to the assays mentioned above. A commercially available antacid formulation (Eno®) was used as the reference drug in each assay. Distilled water and ethanol were used as the negative controls for aqueous and ethanolic extracts respectively.

Results: Both aqueous and ethanolic extracts of *E. alsinoides* exert significant antacid activity demonstrated by the acid neutralising effect and neutralising capacity assays compared to respective controls ($p<0.05$). Acid neutralisation of the aqueous extract was significantly higher than the ethanolic extract ($p<0.05$). Formulated effervescent granules demonstrated significant neutralising activity compared to distilled water in each *in vitro* assay ($p<0.001$). Further, the neutralising capacity assay showed the concentration-dependent activity of the granules. Vatier's stomach model which measures the duration of consistent acid neutralisation, demonstrated the significant activity of the formulation ($p<0.001$).

Conclusion: Aqueous and ethanolic extracts of the *E. alsinoides* plant bear significantly higher antacid activities compared to respective negative controls ($p<0.05$). Effervescent granules formulated using *E. alsinoides* aqueous extract result in a significant *in vitro* antacid effect in all the assay models employed in this study.

Keywords: Antacid, Artificial stomach model, Effervescent, *Evolvulus alsinoides*

INTRODUCTION

Gastric ulceration is an inflammatory condition in which the gastric mucosal cell lining is inflamed due to various aggravating factors. Common aggravating factors are chronic use of alcohol, prolonged use of non-steroidal anti-inflammatory drugs, and *Helicobacter pylori* infection. (1) Some medications used in the treatment of gastric inflammation aim to reduce the acid secretion in the stomach thereby reducing the acidity inside the gastric lumen and promoting gastric ulcer healing. (2) The medicines used to treat gastric ulceration include antacid compounds such as aluminium and magnesium hydroxide, histamine-2 receptor blockers, and proton pump inhibitors (3) At present there is an increased demand for complementary and traditional medicines due to their advantages and benefits. (4)

Evolvulus alsinoides Linn. is a highly valued medicinal plant belonging to the plant family Convolvulaceae. (5) It is commonly known as 'Vishnukranthi' in Ayurvedic medicine and similar vernacular names are used in different parts of South Asia.(6) Despite being a popular medicinal plant with a well-known neuropharmacological action, the whole plant of *E. alsinoides* is widely used in traditional systems for the treatment of fever, asthma, chronic bronchitis, azoospermia, and especially for the treatment of gastrointestinal diseases such as dysentery and gastritis. (7) The plant is an annual or perennial herb which bears blue coloured flowers in its prostrate branches. (8)

A wide array of bioactivities has been tested on different solvent extracts of *E. alsinoides* as reported by different studies. (9–12) The antibacterial activity of the plant had been studied using ethanolic and aqueous extracts against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, and *Escherichia coli*, where a higher microbial growth inhibition had been observed with the ethanolic extract compared to the aqueous plant extract.(9) Furthermore, the ethanolic extract of the whole plant had shown significant anti-inflammatory, antipyretic and anti-diarrhoeal properties in a rat model. (10) Hydroalcoholic extract of the plant had shown strong adaptogenic and memory-enhancing properties in rats. (11) Importantly, the *in vivo* gastroprotective activity of the plant powder has been revealed by a study done on Wistar rats using doses recommended in Ayurveda medicine to treat peptic ulcers. This study has shown that *E. alsinoides* powder is well tolerated by rats without renal or hepatotoxicity, even in chronic use. (12) Indian varieties of *E. alsinoides* are found to contain alkaloids, carbohydrates, phenolic compounds, tannins, saponins and many other phytoconstituents. (8,13) Quantitative analysis of phytochemicals of the Sri Lankan variety of *E. alsinoides* has also been reported in a previous study. (14) High-performance thin-layer chromatographic analysis had indicated the presence of different phytochemicals in the ethanolic extract of the Indian *E. alsinoides* variety. (15)

In the present study, a herbal oral dosage form was proposed to be formulated using the *E. alsinoides* plant extracts. Essentially, an effervescent dosage form was planned in this manner considering their easy reconstitution process, higher palatability, and good patient compliance which have made them have a high demand in the market. (16)

METHODS

Sample collection and authentication

Healthy plants of *E. alsinoides* were collected from Kurunegala (7.7372N, 80.1260E) and Gampaha (7.1621N, 79.8911E) districts of Sri Lanka using standard procedures. (17) The plant materials were air-dried for two weeks and prepared into dry herbarium

specimens which were then authenticated by the National Herbarium, Peradeniya, Sri Lanka.

Preparation of test solutions

Air-dried whole plants of *E. alsinoides* were ground into coarse powder using an electric grinder (Waring Laboratory, United States). Then, a 16.0 g aliquot of the plant powder was refluxed at 100 ^{0}C with 400.0 mL of distilled water for 4 hours. Extracts were cooled to room temperature and then subjected to gravity filtration and subsequently concentrated using a rotary evaporator (Buchi, R 216, Switzerland). Similarly, a 10.0 g aliquot of powdered plant material was refluxed at 70 ^{0}C with 100.0 mL of ethanol for 1 hour, filtered and then the filtrate was concentrated to obtain the ethanolic extract. A commercially available effervescent granular product (Eno®) was used as the positive control and prepared according to the dosing instructions given on the product label. In brief, 5.0 g of the product granules were dissolved in 150.0 mL of distilled water at room temperature and used freshly in each experiment. Distilled water and ethanol were used as the negative controls of the study.

Preparation of artificial gastric acid

All the glassware were rinsed with tap water followed by deionised water and then air-dried adequately. A 3.20 mg quantity of pepsin, 2.00 g of sodium chloride, and 7.0 mL of concentrated hydrochloric acid were added into a 1000 mL volumetric flask and dissolved in an adequate amount of distilled water. The solution was adjusted to pH 1.2 using the electronic pH meter (Thermo Scientific, Eutech pH 6+, China). (18)

Neutralising effects of aqueous and ethanolic extracts of *Evolvulus alsinoides*

Freshly prepared aqueous and ethanolic extracts of *E. alsinoides*, positive control solution, ethanol, and distilled water were taken separately in 9.0 mL quantities and their initial pH values were measured. To each of these samples, 10.0 mL volumes of artificial gastric acid were added separately. Each plant extract was placed in an electronic shaker and their pH values were recorded at 10 minutes intervals until no pH change was observed. The ultimate pH drop (pH difference occurring in the test sample) was reported as the measure of the acid-neutralising effect. (18)

Neutralising capacities of aqueous and ethanolic extracts of *Evolvulus alsinoides*

Initial pH values of freshly prepared aqueous and ethanolic extracts of *E. alsinoides*, positive control solution as well as of ethanol and distilled water were measured. A clean, glass burette was rinsed with deionised water and then filled with fresh artificial gastric acid. A magnetic stirrer was placed in the test solution and the temperature of the solution was set to 37 ^{0}C using an electronic hot plate. The test solutions were titrated with artificial gastric acid until reaching the value of pH 3.0 while being stirred at 20 rpm. The amount of H^+ ions consumed was calculated using the volume of artificial gastric acid consumed in the titration. (19,20)

Preparation of the novel effervescent granules

The aqueous extract of *E. alsinoides* was freeze-dried (LyoBeta, Telstar, Spain) to obtain a dry powder. Wet granulation method was employed to formulate effervescent granules which contained freeze-dried *E. alsinoides* (Figure 1). The composition of the formulation is represented in Table 1. All the excipients except polyvinylpyrrolidone (PVP) were passed through a 150 μm mesh-size sieve and then was mixed with the freeze-dried powder. This mixture was then mixed portion-wise with PVP (pre-mixed with ethanol) while kneading manually.

Figure 1. Formulated *Evolvulus alsinoides* effervescent granules

Table 1. Ingredients of the formulation of effervescent granules

Ingredient	Quantity per dose (g)	Quantity for a one batch (g)
Freeze dried *E. alsinoides* plant powder	0.41	4.05
Tartaric acid	2.93	29.27
Talc powder	0.14	1.39
Magnesium stearate	0.07	0.69
Saccharine	1.36	13.56
Polyethylene glycol	0.22	2.21
Citric acid	1.46	14.64
Sodium bicarbonate	4.98	49.76
Polyvinylpyrrolidone	0.44	4.43
Ethanol	q.s.	q.s.
Total	**12.00**	**120.00**

The wet dough obtained thereafter was passed through a 2000 μm mesh-size sieve to form wet granules. These granules were then dried in a hot air oven (SANYO, MIR 162, Japan) at 40 ^{0}C for 4 hours. Finally, the dry granules were transferred into air-tight polythene packaging containers and stored in a refrigerator. (21,22)

Determination of *in vitro* antacid activity of effervescent granules

A series of suspensions were prepared using the formulated effervescent granules in different concentrations ranging from 0.006 – 1.0 g/mL. *In vitro* acid neutralising activities of these test samples were then assayed against the fresh artificial gastric acid according to the methods described above. Additionally, modified Vatier's artificial stomach model was used to determine the duration of consistent neutralisation of the artificial gastric acid. The artificial stomach model comprised a gastric acid reservoir, a tubing system that secretes and excretes artificial gastric acid into the reservoir, and a motor system, pumping the acid. Magnetic stirrers placed separately in beakers with effervescent test matter, positive control (Eno®) or distilled water were set to rotate at 30 rounds per minute speed whereas the temperature of the solution was set to 37 ^{0}C. The time taken by each test solution to achieve a value of pH 3.0 while the tube-motor system simultaneously secretes and excretes gastric acid at a 3.0 mL/minute rate was measured in the assay. (18–20,23)

Statistical analysis

All the experiments were triplicated (n=3) and results were recorded as mean ± standard error of the mean (SEM). Test samples were statistically compared with respective control samples by the SPSS 25.0 software. Analysis

of variance (ANOVA) was performed and was followed by Tukey's post hoc test.

RESULTS

Neutralising effects of aqueous and ethanolic extracts of *Evolvulus alsinoides*

Aqueous and ethanolic extract of *E. alsinoides*, reference drug, distilled water and ethanol resulted in respective initial pH values of 5.81±0.00, 4.92±0.02, 6.45±0.01, 7.88±0.01, and 6.85±0.00. According to the mean values of the pH reduction in each test sample shown in Figure 2, both aqueous and ethanolic extracts of *E. alsinoides* demonstrated statistically significant neutralising effects compared to the respective negative controls ($p<0.05$). However, the neutralising effects of both of the solvent extracts were found to be significantly ($p<0.05$) lower compared to the positive control sample, which resulted in the minimum pH drop in the experiment.

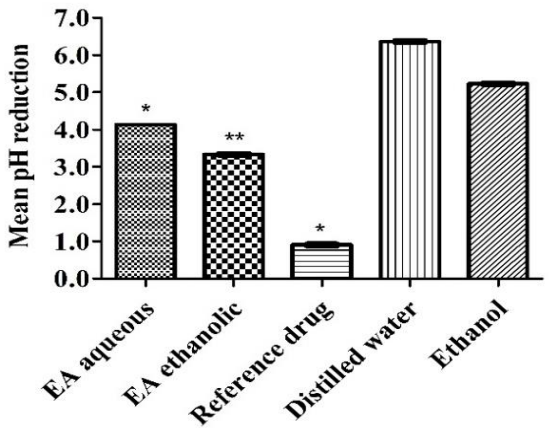

Figure 2. Neutralising effect of aqueous and ethanolic extracts of *Evolvulus alsinoides*

EA: *Evolvulus alsinoides*. Significant *p<0.05 compared to distilled water; **p<0.05 compared to ethanol. Values are presented as mean ± SEM (n=3)

Neutralising capacities of aqueous and ethanolic extracts of *Evolvulus alsinoides*

Table 2 shows the results of the *in vitro* acid neutralising capacity assay. Both aqueous and ethanolic extracts of *E. alsinoides* have significantly higher neutralising capacities compared to the respective negative controls ($p<0.05$). However, the neutralising capacities of both of the solvent extracts were found to be significantly lower ($p<0.05$) compared to the positive control sample, which consumed the highest number of H^+ ions in the assay. When comparing the two solvent extracts, neutralising capacity of the aqueous plant extract was significantly higher than the ethanolic extract ($p<0.05$).

Table 2. Neutralising capacities of aqueous and ethanolic extracts of *Evolvulus alsinoides*

Test sample	Consumed H^+ ions (mmol)
E. alsinoides aqueous extract	0.24±0.00*
E. alsinoides ethanol extract	0.07±0.00**
Eno®	2.07±0.01*
Distilled water	0.01±0.00
Ethanol	0.01±0.00

Significant *p<0.05 compared to distilled water; **p<0.05 compared to ethanol. Values are presented as mean ± SEM (n=3)

***In vitro* antacid activity of effervescent granules**

Initial pH of the effervescent granule test suspensions in concentrations of 0.10, 0.05, 0.025, 0.013, and 0.006 g/mL were pH 8.09±0.05, 7.49±0.01, 6.16±0.01, 6.28±0.01, and 5.87±0.01 respectively. According to the end pH of effervescent granules at different concentrations as shown in Figure 3, all the

test samples exerted statistically significant neutralising effects compared to the negative control ($p<0.001$).

It is noteworthy that all the concentrations of the formulated granules showed significantly higher neutralising capacities compared to distilled water ($p<0.001$). Moreover, effervescent granules demonstrated a dose dependent ($r^2=0.99$) neutralizing capacity (Figure 4).

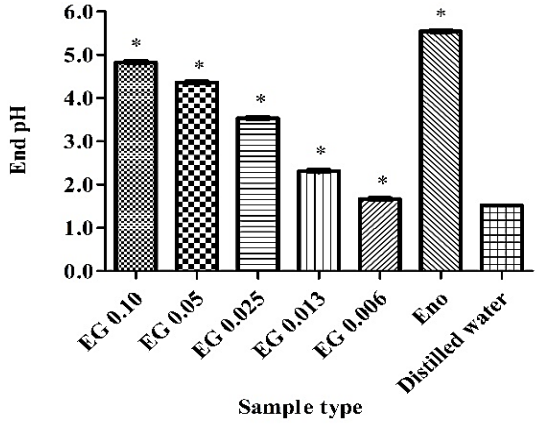

Figure 3. *In vitro* acid neutralising effect of *Evolvulus alsinoides* effervescent granules in different concentrations

EG: Effervescent granules. Significant *p<0.001 compared to the negative control sample of neutralising effect assay. Values are presented as mean ± SEM (n=3)

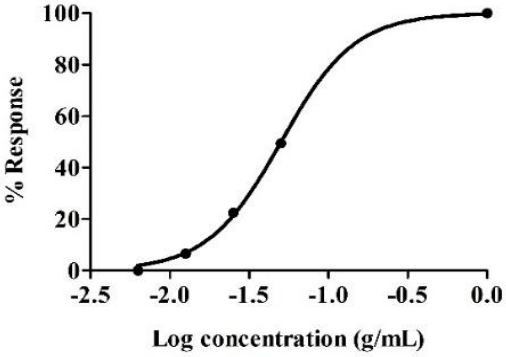

Figure 4. Concentration-dependent acid neutralising capacity of effervescent granules formulated using *Evolvulus alsinoides* powder

Granule concentration that provides 50% neutralisation to the gastric acid sample (EC_{50} of the dosage form) is 0.0502 g/mL. Table 3 shows the time duration taken to achieve consistent neutralisation of the artificial gastric acid by the formulated effervescent granules. Both the tested concentrations showed a significantly different time duration of acid neutralisation, compared to the control ($p<0.001$).

Table 3. Time taken to achieve consistent acid neutralisation *In vitro* by *Evolvulus alsinoides* effervescent granules

Test sample (g/mL)	Time taken to achieve consistent neutralisation (sec)
Effervescent granules (0.05 g/mL)	373.96±22.65[a]
Effervescent granules (0.10 g/mL)	466.44±10.36[a]
Reference drug, Eno® (0.05 g/mL)	453.07±21.00[a]
Distilled water	14.26±5.66

[s]Significant ($p<0.001$) compared to negative control of neutralising duration assay. Values are presented as mean ± SEM (n=3)

DISCUSSION

The development of dosage forms using herbal materials has become a novel scientific trend in tropical Asian and African countries since the social insight of traditional and complementary medical systems has been broadened in the last few decades. (24) This study was carried out to investigate the antacid activity of an important plant species of family Convolvulaceae; *E. alsinoides* which, the confirmation in turn was utilised in the formulation of a dosage form containing extracts of this plant.

Ethnopharmacological literature and previous *in vivo* studies have disclosed the

gastroprotective activity in aqueous extracts and the dry powder of the plant.(10,12) In this study, the *in vitro* acid-neutralising activity of the ethanolic plant extract was studied in addition to the crude aqueous extract, which would be the first-ever record of such activity in the ethanolic extract of the plant *E. alsinoides*. Acid-neutralising activities of both solvent extracts were significantly higher compared to the respective negative controls ($p<0.05$) and at the same time, it was also statistically shown that the activity produced by *E. alsinoides* aqueous extract was significantly higher compared to the ethanolic extract in both neutralising effect and neutralising capacity assays ($p<0.05$). Hence, the pre-formulation of the effervescent dosage form was continued only with the aqueous extract of *E. alsinoides* which has also been proven for gastroprotection on animal models previously. (12)

Gastroprotection provided by different plant materials and extracts may be mediated through different phytochemicals that possess the ability to neutralise gastric acid at different concentrations.(25) Several studies done on Sri Lankan as well as Indian varieties of this plant have already reported the presence of different phytochemicals in aqueous, ethanolic and ethyl acetate extracts. (13,14,26) In general, most of these phytochemical compounds exert slight acidic or basic properties due to the various functional groups they bear in the molecular structure. (27) As an example, phenolic compounds and flavonoids are known to have alkaline properties since the hydroxyl groups that are abundant in their structure can act as electron-donating or electron-accepting sites depending on the pH of the reaction medium. (28) These functional group sites may donate electrons to free protons available in gastric acid and then neutralise the medium. *E. alsinoides* belongs to the plant family Convolvulaceae, which includes plant species containing flavonoids such as kaemferol, quercetin, isorhamnetin and their related flavonoid glycosides. (29) Therefore, considering the chemotaxonomic relevance, it can be postulated that the study plant may also contain important phenolic compounds. Alkaloids are another group of phytochemicals which bears one or more basic nitrogen atoms in their structure. (30) These nitrogen atoms usually occur as amino groups and thus can act as electron-donating sites to neutralise H^+ ions. (31,32) It is well known that the plant family Convolvulaceae is a major source of ergot-type alkaloids, thus the presence of such alkaloids in *E. alsinoides* is also predictable. (33) Since phytochemical evidence for the specific phytoconstituents found in the study plant and details on their precise chemical properties are inadequate, these postulations may also be tested in future pharmacognostic studies elaborating molecular mechanisms.

For the determination of the antacid activity of the crude plant extracts, only two *in vitro* assay methods were conducted in this study though more advanced assay methods are established in research. (18) The modified model of Vatier's artificial stomach is such a method that tests the duration of transient acid-neutralisation given by the test samples. An in-house fabricated stomach model was utilized in the investigation of the antacid activity of the formulated granules. This model is more physiological unlike most of the other *in vitro* assays, as it simulates several biological conditions in the stomach. The temperature in the apparatus is maintained at 37 ^{0}C, which is the body temperature of healthy humans. Gastric acid secretion and peristaltic movements are also imitated by the magnetic stirrer and electronic pump system respectively. (23) It is notable

that only 9.0 mL volumes of samples were used with 10.0 mL of artificial gastric acid in this study despite the literature recommends using 90.0 mL portions of sample solution with 100.0 mL of artificial gastric acid in each test. This minute modification could be compared by appropriate arithmetic justification and may be used in future experiments which involve the evaluation of *in vitro* neutralising activity of low-yield or rare herbal extracts effectively.

Solid pharmaceutical preparations are chemically and microbiologically more stable than liquid preparations, acknowledging their reduced moisture contents. (22) Oral granules are a more convenient dosage form to dispense a large solid medicine content at once as a single dose. (34) Moreover, the oral granules which are formulated by incorporating a mixture of citric acid and sodium bicarbonate produce carbon dioxide bubbles during the reconstitution. This modification made the granules effervescent and may result in an oral dosage form with improved patient compliance to the dosage regimen. (35) The quick onset of action is another advantage of these effervescent granules compared to conventional solid dosage forms. (36) Although the dosage form is manufactured as a solid, it is administered in liquid form. Gastric hyperacidity and related functional dyspepsia have become more common diseases conditions, where nearly 20 % of the world wide population is known to suffer from. (37) Moreover, several classes of western medicines that are widely used to treat gastric disorders are known to have a wide range of unpleasant side-effects. (38) Hence, fast-acting, herbal antacid formulations with fewer side-effects are needed for the management of such disease conditions more efficiently and cost-effectively. Regarding the formulation process, saccharine and tartaric acid were used as the sweetening agent and flavouring agent (grape-like taste) respectively. To make the granules effervescent, a mixture of solid sodium citrate and sodium bicarbonate was incorporated in minute amounts into the dosage form. Magnesium stearate and talc were used in the formulation process to obtain their conventional properties as lubricants whereas PVP was used as the binding agent. (21,39) It should be mentioned that ethanol was used in the preparation of the polyvinylpyrrolidone contained binding solution. Since ethanol itself has been reported to cause gastric irritation, formulated granules were dried well to evaporate all the ethanol residues in the powder blend. (40) For a follow-up study, it is recommended to conduct an assessment to determine the absence of ethanol in the formulation. Major limitation of this study was the incorporation of sodium bicarbonate, which is a weakly alkaline chemical compound. For the preparation of effervescent dosage form, incorporation of sodium bicarbonate was essential and omission of this substance would lead to lose of the effervescent characteristic. However, the quantity of bicarbonate used in the study was thoroughly limited and incorporated bicarbonate was intended to be completely reacted with citric acid before the administration. Further, the reference drug Eno® itself contain sodium bicarbonate and bicarbonate was incorporated into the present dosage form only as an excipient which would not involve in its biological actions. Further, preclinical animal trials are suggested to evaluate the efficacy of the effervescent granules along with suitable dose-determination studies.

CONCLUSION

E. alsinoides plants in aqueous and ethanolic extracts bear significantly higher *in vitro* antacid activities compared to respective

negative controls. The activity was found to be lesser than the reference drug. The ethanolic extract possesses less activity than the aqueous extract in both acid-neutralising effect and neutralising capacity tests. Effervescent granules formulated using *E. alsinoides* aqueous extract resulted a significant acid neutralisation in all the assay models, paving the way for the bioactivity to be investigated in future *in vivo* studies.

Author's declaration:
The authors declare that all persons listed as authors have read and given approval for the submission of this manuscript.

Competing interests:
The authors declare that they have no competing interests to disclose.

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