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In vitro Antacid Screening of the Aqueous and Ethanolic Leaf extracts of *Triticum aestivum* (Linn.) and *Hordeum vulgare* (Linn.)

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ABSTRACT

Triticum aestivum L. (wheat grass) and *Hordeum vulgare* L. (barley grass) are functional foods with numerous pharmacological properties. Crude aqueous and ethanolic leaf extracts of *T. aestivum* L. (wheat grass) and *H. vulgare* L. (barley grass) were screened for *in vitro* antacid activity using the preliminary antacid test, determination of acid neutralization capacity, acid neutralizing effect, duration of consistent neutralization, and buffering capacity. Results of the preliminary antacid test showed that the aqueous extracts had better antacid potential than the ethanolic extracts. Among the extracts, *T. aestivum* aqueous extract exhibited the most potent *in vitro* antacid activity, with acid neutralization capacity of 0.0763±0.0028 mmol H^+, acid neutralizing effect 0.043±0.006 ΔpH, duration of neutralization 22±1.732 min, and buffering capacity of 0.0801±0.0331 mmol H^+/ΔpH. Alkaloid content of each extract was also determined gravimetrically; the *T. aestivum* ethanolic extract had the highest amounts of alkaloids (16.8518 ± 2.5368%). This study provides proof of the antacid activities of ethanolic and aqueous extracts of *T. aestivum* and *H. vulgare*, with the aqueous *T. aestivum* extract the most active.

Keywords: *Triticum aestivum*, *Hordeum vulgare*, antacid potential.

INTRODUCTION

Peptic ulcer is one of the occupational diseases of employees in non-agricultural establishments in the Philippines, with 5, 347 cases in 2003 and 4, 135 cases in 2007[1]. Due to the high prevalence of peptic ulcer disease, there is an increasing demand for different anti-ulcer drug. In fact, almost 15,000 deaths occur each year due to peptic ulcer disease[2]. Antacids are weak bases that are capable of reacting with gastric acid to form water and salt, thereby reducing gastric acidity. Commonly used antacid preparations contain one or a combination of the following alkaline active ingredients:calcium carbonate, sodium bicarbonate, aluminum hydroxide, and magnesium compounds

such as magnesium hydroxide[3]. Although proven to be effective, prolonged exposure to these drugs often leads to serious adverse effects such as constipation, diarhea, and even chronic renal failure[4]. Therefore, there is a need for an alternative that will provide the same beneficial effects but with less adverse effects.

Because of this, many researchers are now seeking for natural ways of combatting these stomach disorders as well as their symptoms. *Triticum aestivum* L. and *Hordeum vulgare* L. are gaining popularity due to their wide range of pharmacological activities. *T. aestivum* or wheat grass is termed as "functional food" due to its various health benefits: as dietary supplement and treatment of minor ailments[5], and various conditions such as anemia, diabetes, cancer, kidney swelling, and common cold. It was also observed to minimize fatigue, increase strength, regulate blood pressure and blood sugar, support weight loss, improve digestion, improve mental function, decrease rate of cellular aging[6], and heal ulcers[7]. *H. vulgare* or barley is a widely consumed cereal and considered as the fourth most important cereal crop next to wheat, maize, and rice, being cultivated worldwide in all non-tropical countries. Barley is considered as the most useful grain due to its digestibility and chemical constituents. Based on the research findings of Kazuhiko Kubota, barley leaf extracts possess many pharmacological functions which include anti-inflammation, anti-ulcer[8], anti-hypercholesterol, anti-thrombosis, anti-anxiety, increase in edurance, as well as lowering of blood sugar[9].

The present study aims to evaluate the *in vitro* antacid activities of *T. aestivum* and *H. vulgare* ethanolic and aqueous extracts using the preliminary antacid test and determination of acid neutralization capacity, neutralizing effect, duration of consistent neutralization and acid buffering capacity. It also includes phytochemical screening, focusing on the amount of alkaloids in the plant extracts.

MATERIALS AND METHODS

Materials and reagents

Fresh leaves of *T. aestivum* and *H. vulgare* were purchased from Quezon Memorial Circle Plant Center (Quezon City, Philippines). Voucher samples were submitted to the National Museum of the Philippines for proper authentication and safekeeping.

Solvents and chemicals used in the study were technical grade (Belman, Philippines; Sigma Aldrich, Singapore). All pH measurements were determined using the Ohaus Starter 300.

Preparation of Artificial Gastric Juice and Stomach Model

The artificial gastric juice was prepared according to the simulated gastric fluid test solution by the United States Pharmacopeia (USP)[10]. This dissolution media simulates the gastric juice during the fasted state[11]. Two grams of sodium chloride and 3.2 mg of pepsin were dissolved in 7 mL of concentrated hydrochloric acid (12 M) and diluted with deionized water to make a 1 liter solution of the artificial gastric juice at pH 1.2. The artificial gastric juice was stored at 4 °C until further use.

The artificial stomach model used for the determination of duration of consistent neutralization consisted of three elements: a pH recording system, a stomach, and a micro-tubing peristaltic pump (Fig. 1). The stomach is made up of three portions, S1, S2, and S3. S1 serves as a reservoir containing the treatment solution and artificial gastric juice, whereas S2 and S3 are used to simulate the secretory and gastric emptying fluxes, respectively[12].

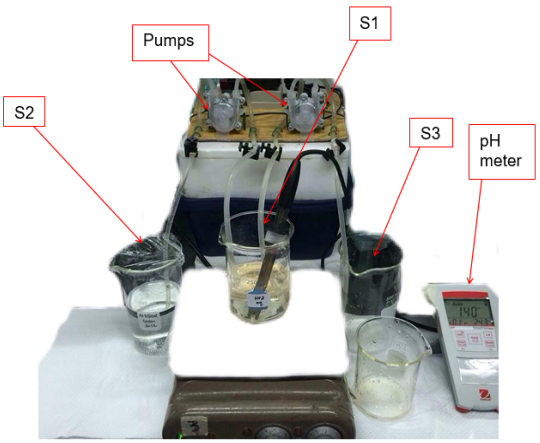

Fig. 1. The artificial stomach model consists of three elements: peristaltic pump, pH meter and stomach. The stomach consists of three elements: S1 represents the reservoir containing the treatment solution and artificial gastric juice, and S2 and S3 represent the gastric secretory and emptying fluxes, respectively.

Preparation of Crude Extracts

Fresh leaves of *T. aestivum* and *H. vulgare* were air dried at room temperature for several days and then ground. About 100 g (dry weight) each was macerated (1:5, w/v) four times with technical grade ethanol at room temperature for 72 h with occasional shaking using a mechanical shaker and with deionized water at 4 °C for 24 hours. The samples were extracted five times to ensure complete extraction, then filtered, and concentrated using rotary evaporator for the ethanolic extracts and lyophilization for the aqueous extracts. The crude extracts were stored at -41 °C until further use.

***In Vitro* Antacid Screening: Preparation of Test Solutions**

Stock solutions (100 mg/mL) of the ethanolic and aqueous extracts of *T. aestivum and H. vulgare* were initially prepared using absolute ethanol as solvent for the ethanolic extracts and deionized water for the aqueous extracts. From the stock solution, a final diluted concentration of 1 mg/mL was prepared in triplicates. In the case of the ethanolic extracts, the final concentration was 1 mg/mL in 1% ethanol. Sodium bicarbonate (1 mg/mL in 1% ethanol) was used as the positive control. For the aqueous extracts, deionized water was used as solvent and the positive control was also sodium bicarbonate (1 mg/mL) in deionized water. Deionized water was used as the negative control for the aqueous extracts and 1% ethanol for the ethanolic extracts. For each experiment, the setups were maintained at 37 °C.

Preliminary Antacid Test

Preliminary antacid test was perfomed based on the method stated in the United States Pharmacopeia (U.S.P) national formulary[10]. Forty milliliters (40 mL) of each test solution was continuously stirred for 1 minute. Then, 10 mL of standardized 0.5 M HCl was added. The mixture was continuously stirred for 10 minutes and then the pH was measured.

Determination of the Acid Neutralization Capacity (ANC) Using the Titration Method of Fordtran's Model.

Acid neutralization capacity was determined using the titration method of Fordtran's model[12,13]. Fifty milliliters (50 mL) of each test solution was continuously stirred and then titrated with artificial gastric juice to the endpoint of pH 3. The total consumed hydrogen ions (mmol H^+) was calculated by multiplying the concentration of artificial gastric juice used by its volume that was added to the sample.

Determination of the Neutralizing Effect of Extracts on Artificial Gastric Juice

Fifty milliliters (50 mL) of each test solution was added to 55 mL artificial gastric juice at pH 1.2. The resulting pH value was determined.

Determination of the Duration of Consistent Neutralization Using a Modified Artificial Stomach Model

Fifty milliliters of each test solution (1 mg/mL) was added to 55 mL of artificial gastric acid juice at pH 1.2 in the reservoir (S1) of the artificial stomach at 37 °C and was continuously stirred at 60 rpm. Artificial gastric juice at pH 1.2 was pumped at 3 mL/min. into S1 of the artificial stomach, and simultaneously pumped out at 3 mL/min. pH changes were determined in S1 in three minute intervals. The duration of neutralization was determined when the pH value has returned to its initial value (pH 1.2).

Acid-Buffering Capacity Assay

Buffering capacity was evaluated based on the Official Methods of Analysis of the Association of Official Analytical Chemists[14]. Forty milliliters (40 mL) of each test solution was continuously stirred at 60 rpm. Forward titration was performed by gradual addition of 0.5 mL of standard 0.099207 M HCl until the pH decreased to 1.5, *i.e.* Normal stomach pH. The samples were then back titrated by gradual addition of 0.5 mL of standard 0.100995 M NaOH until the pH increased to 10. Initial pH level and all further measurements taken during titration were recorded after an equilibration period of 1 min. after addition of acid or base. The acid buffering capacity (BC) was computed based on the equation by Van Slyke: total volume of acid added to each sample multiplied by the acid molarity, divided by the total change in pH[15].

Phytochemical Analysis

The ethanolic and aqueous extracts of *T. aestivum* and *H. vulgare* were screened for the presence of different phytochemicals such

as flavonoids, alkaloids, tannins, indoles, anthraquinones, and anthrones using the standard tests in Table 1[16].

Table. 1. Phytochemical tests

Phytochemical	Spray test
Flavonoids	Antimony(III) chloride
Tannins	Potassium-ferricyanide-ferric chloride
Alkaloids	Dragendorff's reagent
Anthraquinones, anthrones	Methanolic potassium hydroxide (Börntrager reagent
Indoles	Van Urk-Salkowski reagent

Determination of Percent Alkaloid Content

Total alkaloid content was determined gravimetrically using the method of Harborne[17]. Two grams of the extract was weighed and dissolved in 80 mL of 10% acetic acid in ethanol. The mixture was shaken and incubated at room temperature for 4 h before it was filtered. The filtrate was concentrated to ¼ of its original volume. Concentrated ammonium hydroxide was added dropwise until precipitation was complete. The solution was filtered and the filter paper containing the precipitate was dried in an oven at 60 °C for 30 min. and then cooled in a dessicator. The final weight was recorded and the total alkaloid content was expressed as a percentage of the sample weight analyzed. The results of the percent alkaloid content were correlated to the results of each *in vitro* antacid test.

Statistical Analysis

Data were expressed as mean value ± SD of three replicate measurements. Data analyses were carried out using Microsoft Excel 2013. Statistical analyses were carried out using IBM SPSS Statistics 20. The significance of the differences between controls and test solutions was analyed using analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. Differences at $p<0.05$ were considered to be significant ($\alpha=0.05$).

RESULTS AND DISCUSSION

Extraction, initial pH values and preliminary antacid test

After complete extraction of the samples, the percent yields were found to be: *T. aestivum* ethanolic (19.49%) and aqueous (27.98%), and *H. vulgare* ethanolic (17.99%) and aqueous (29.78%).

Table. 2 shows that the ethanolic extract of *T. aesitvum* had the higher pH (pH=4.92) compared to the ethanolic extract of *H. vulgare.* The aqueous extract of *H. vulgare* on the other hand, had higher pH (pH=6.17) than that of *T. aesitvum.* The preliminary antacid tests on the plant extracts showed higher pH values compared to the negative controls for *T. aestivum* ethanolic ($p=0.027$, $\alpha=0.05$), *H. vulgare* ethanolic ($p<0.0001$, $\alpha=0.05$), *T. aestivum* aqueous ($p=0.004$, $\alpha=0.05$), and *H. vulgare* aqueous extracts ($p=0.007$, $\alpha=0.05$). The preliminary antacid test was used to determine if a single dose of a substance is capable of increasing the pH of a 10 mL solution of 0.5 M HCl to greater than or equal to 3.5 after 10 min. of reaction, as stated in the United States Pharmacopeia national formulary; substances that satisfy this criterion are considered as "antacids"[10]. It can be observed that the results of the modified preliminary antacid test agree with the

Table. 2. Initial pH values of the negative control, positive control, and plant extracts (1 mg/mL). Preliminary antacid test results of negative and positive controls, and plant extracts. Result shown is average pH ± SD

Sample	pH		Preliminary antacid test (pH)	
	Ethanolic	Aqueous	Ethanolic	Aqueous
Negative Control*	5.62	5.67	0.9879±0.0023	0.9784±0.0025
$NaHCO_3$ (1 mg/mL)	8.56	8.54	1.0389±0.0008°	1.0655±0.0045°
Triticum aestivum	4.92	5.43	0.9944±0.0025°	0.9966±0.0015°
Hordeum vulgare	4.82	5.62	0.9902±0.0008	0.9957±0.0020°

*Negative Control: Ethanolic (1% Ethanol); Aqueous (Deionized Water) °$p<0.05$ vs negative control. The mean difference is significant at the 0.05 level.

respective initial pH values. *T. aestivum* had the highest initial pH value among the ethanolic extracts, and all of the aqueous extracts had even higher initial pH values, thereby explaining their observed significant increase in pH versus the respective negative controls. Furthermore, it can be observed that for all plant samples, the aqueous extracts had consistently higher antacid potential than the ethanolic extracts, although still significantly lower than that of the standard solution of sodium bicarbonate ($p<0.0001$, $\alpha=0.05$).

Acid Neutralization Capacity, Acid Neutralizing Effects on Artifical Gastric Juice, Duration of Neutralization

Table.3 shows the acid neutralization capacities, the neutralizing effects of the ethanolic and aqueous extracts on gastric juice, and the duration of consistent gastric acid neutralization. For the ethanolic extracts, Tukey's Post Hoc Test revealed that the acid neutralization capacities (ANC) of *T. aestivum* was not signifantly higher ($p=0.268$, $\alpha=0.05$) than *H. vulgare*, albeit higher. On the other hand, for the aqueous extracts, *T. aestivum* had a significantly higher ANC than *H. vulgare* ($p<0.0001$, $\alpha=0.05$). Aside from the preliminary antacid test, the acid neutralization capacity (ANC) is often used to evaluate the effectiveness of different antacids. Acid neutralization capacity is the amount of hydrochloric acid an antacid can neutralize, expressed as mmol of H^+[18].

The acid neutralizing effects on artificial gastric juice results are also shown in Table. 3. Tukey's Post Hoc Test revealed that both ethanolic extracts showed a significant increase in pH when compared to the negative control ($p<0.0001$, $\alpha=0.05$, for both extracts), although significantly lower than that of the positive control ($p<0.0001$, $\alpha=0.05$, for both extracts). On the other hand, for the aqueous extracts, both crude aqueous extracts showed a significant increase in pH ($p<0.0001$ for *T. aestivum*; $p=0.011$ for *H. vulgare*, $\alpha=0.05$) when compared to the negative control. The neutralizing effect on artificial gastric juice can be used as a measure of the onset of action of antacids since in this case, the resulting pH is directly determined upon addition of the sample solution to a fixed volume of the artificial gastric acid. It is an important factor and must be taken into account when evaluating antacid potential since one criterion of an ideal antacid is that it must react rapidly with acids[19]. The ethanolic extracts of *T. aestivum* and *H. vulgare* showed potent neutralizing effects.. The neutralizing effect, however, was higher for the aqueous extract of *T. aestivum.* These observations are consistent with those observed in the acid neutralization capacities of the extracts.

On the test for the duration of neutralization (Table. 3), the ethanolic extract of *T. aestivum* showed longer duration than the ethanolic extract of *H. vulgare* ($p=0.977$, $\alpha=0.05$). However, the

Table. 3. Acid neutralization capacity (ANC), acid neutralizing effect on gastric juice and duration of neutralizing effect of the negative and positive controls and plant extracts. Results shown as average ANC ± SD.

Sample	Acid Neutralization Capacity (mmol H^+)		Acid Neutralizing Effect on Gastric Juice (%Acid Neutralized)		Duration of Neutralization (min.)	
	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous
Negative control*	—-	—-	—-	—-	—-	—-
$NaHCO_3$ (1 mg/mL)	0.8297± 0.0006	0.8030± 0.0006	16.197°	13.176°	28±1.732°	28±1.732°
Triticum aestivum	0.0513± 0.0004	0.0763± 0.0028	3.996°	5.592°	13±1.732°	22±1.732°
Hordeum vulgare	0.0490± 0.0015	0.0513± 0.0016	3.996°	2.650°	12±1.732°	11±0.000°

*Negative Control: Ethanolic (1% Ethanol); Aqueous (Deionized Water) °$p<0.05$ vs negative control. The mean difference is significant at the 0.05 level.

aqueous extract of *T. aestivum* exhibited a significantly longer duration of action than the *H. vulgare* extract ($p<0.0001$, $\alpha=0.05$). The duration of consistent gastric acid neutralization was evaluated using a modified model of Vatier's artificial stomach which mimics the regular physiological functioning of the human stomach[12]. In this model, only the interaction between the plant extract and the artificial gastric juice simulating the fasted state was monitored, and therefore, interaction with food particles was not included in the study. The secretion or gastric acid introduction and emptying rates were set at 3 mL/min, the rate that lies within the range for the peak acid output in both males and females.

Acid Buffering Capacity Assay

Since the aqueous extracts exhibited consistently higher antacid activities than the ethanolic extracts, only the aqueous extracts were screened for their buffering capacities.

Table. 4 shows the buffering capacities of the aqueous extracts, as well as that of the standard solution of sodium bicarbonate and deionized water. As observed, *T. aestivum* exhibited higher buffering capacity. This is consistent with the results of the Tukey HSD Post Hoc Test that showed *T. aestivum's* buffering capacity to be not significantly different from that of sodium bicarbonate ($p=0.627$, $\alpha=0.05$), thereby implying that the aqueous extract of *T. aestivum* is capable of buffering gastric acid to an extent that is comparable to that of sodium bicarbonate. It can be observed that for both the acid neutralization and acid buffering capacities, the aqueous extract of *T. aestivum* showed the highest activity.

Phytochemical Screening and Alkaloid Quantification

Phytochemical analysis of the crude ethanolic and aqueous extracts of the plant samples was also performed (Table. 5). The aqueous extracts of *T. aestivum* and *H. vulgare* tested negative for flavonoids, anthraquinones, and anthrones. Alkaloids, indoles, and tannins were found to be present in all of the crude ethanolic and aqueous extracts. The presence of alkaloids could have possibly contributed to the antacid potential of the extracts.

Pharmacological properties of alkaloids include analgesic, central nervous stimulant and depressant, antihypertensive, anticholinergic, antitumor, antimalarial activities[20], as well as anti-ulcer activity[21]. These compounds can react with acid to form crystalline salts without producing water[22]. Hence, the correlation between the percent alkaloid content of each extract (in % g alkaloid/g plant material) and the results of the *in vitro* antacid activities was determined in this study.

The total alkaloid content of the ethanolic and aqueous extracts was determined gravimetrically using the method of Harborne[17]. The results are as follow: *T. aestivum* ethanolic extract = 16.8518 ± 2.5368% w alkaloid/w extract; *H. vulgare* ethanolic extract = 15.0244 ± 1.6594% w alkaloid/w extract; *T. aestivum* aqueous extract = 12.6025 ± 1.7135% w alkaloid/w extract; and *H. vulgare* aqueous extract = 9.9112 ± 1.2394% w alkaloid/w extract. The high alkaloid content of the extracts could have contributed to the buffering capacities by virtue of their reaction with HCl to form their corresponding salts.

Table. 4. Acid buffering capacity of positive control and aqueous plant extracts (1 mg/mL). Acid buffering capacity was determined by dividing the titratable alkalinity (mmol H+) by the total change in pH units

Sample	Acid Buffering Capacity (mmol H^+/pH unit)
Negative control*	—-
$NaHCO_3$ (1 mg/mL)	0.1060 ± 0.0082°
Triticum aestivum (aqueous)	0.0801 ± 0.0331°
Hordeum vulgare (aqueous)	0.0356 ± 0.0038

*Negative Control: Ethanolic (1% Ethanol); Aqueous (Deionized Water) °$p<0.05$ vs negative control. The mean difference is significant at the 0.05 level.

Table. 5. Phytochemical tests of the crude aqueous and ethanolic extracts of *Triticum aestivum* and *Hordeum vulgare*

Plant sample	Flavonoids	Alkaloids	Indoles	Tannins	Anthra-quinones	Anthrones
T. aestivum ethanolic	+	+	+	+	+	+
T. aestivum aqueous	-	+	+	+	-	-
H. vulgare ethanolic	+	+	+	+	+	+
H. vulgare aqueous	-	+	+	+	-	-

*Legend: '+' = positive reaction, '-' = negative reaction.

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