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PHYTOCHEMICAL ANALYSIS AND EFFECT OF EXTRACTING SOLVENTS ON THE *IN VITRO* ANTACID ACTIVITY BY AN ARTIFICIAL GASTRIC ACID MODEL OF LEAF EXTRACTS OF *Ruellia tuberosa* L., Acanthaceae

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ABSTRACT

In the traditional medicine of some Asia and other countries, Ruellia tuberosa L. was used commonly with pharmacology activities such as gastric ulcers, anti-pyretic, analgesic, anti-hypertensive and so on. However, this plant got little attention in Viet Nam. On that basis, this paper primarily studied on phytochemical constituents and in vitro antacid activity by an artificial gastric acid model of leaf extracts of Ruellia tuberosa L.. The methanol extract was identified of 18 compounds when analyzing by GC-MS. In fact, Ar-turmerone (26.35%), β-Sitosterol (21.64%), Silanediol, dimethyl-,diacetate (9.18%), Curlone (7.59%), Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene- (7.54%) were the chief chemical compounds. In addition, in vitro antacid activity on artificial gastric acid of Fordtran's model was also conducted in this study. The results showed ethanol 50% extract had the strongest neutralizing activity; effect of the other extracts were arranged in descending order in the following: water extract, ethanol 70% extract, methanol extract, and ethanol 96% extract. This demonstrated that the extracting solvents significantly affect on neutralizing activity of leaf extracts of Ruellia tuberosa L. It expected that the 50% ethanol leaf extract of Ruellia tuberosa L. would be a potential antacid product with few side effects and drug interactions.

1. INTRODUCTION

Ruellia tuberosa Linn. belongs to family Acanthaceae, a native of Central America; It is used medicinally in West Indies, Central America, Guiana, and Peru. In many countries, it was known as "cracker plant" is traditionally used as diuretic, anti-pyretic, analgesic, anti-hypertensive, anthelmentic, abortifacient, emetic, in bladder disease, kidney disorder, bronchitis, gonorrhoea and syphilis (Daya L.C. *et al.*, 2010). Especially, in traditional medicine of India, *Ruellia tuberosa* L. has been employed to treat gastrointestinal disorders including gastric ulcers (Sathyavathi G.V. *et al.*, 1987). Many scientific references proved on phytochemistry and pharmacology of *Ruellia tuberosa* L. in the world (Daya L.C. *et al.*, 2010). Although it was a common plant in Viet Nam, there is almost no study on chemical compositions and pharmacology activities of *Ruellia tuberosa* L. with the best of our knowledge. On that basis, this paper primarily

studied on phytochemical constituents and *in vitro* antacid activity of leaf extracts of *Ruellia tuberosa* L..

2. MATERIALS AND METHODS

2.1 Materials

Fresh leaves of *Ruellia tuberosa* L. were collected in Vinh Chau district, Soc Trang province in Aug 2018. After that, they were washed, chopped, dried in shade, and ground to powder as raw materials used in this study.

Chemicals and reagents

Sodium chloride and pepsin were purchased from Sigma (St. Louis, MO, USA) and 1 mol/L hydrochloric acid was obtained from Merck (Darmstadt, Germany). The active control drugs such as Sodium Bicarbonate (SB), including 500 mg Sodium Bicarbonate (Binh Dinh Pharmaceutical Co. Ltd) and Edoz Kids Powder, including 800 mg Sodium Bicarbonate and the other ingredients (Duoc Hau Giang Pharmaceutical Co. Ltd).

2.2. Methods

2.1.1 Identification of phytochemical from methanol extract by GC-MS analysis

Preparation of the methanolic extract

A portion of dried leaves (200 g) of *Ruellia tuberosa* L. was macerated with 750 ml of methanol for 48 h at room temperature. This extract was filtered through a 45 µm filter. The resulting solution was concentrated in vacuum to dryness to give methanol extract (9 g).

GC-MS (Gas Chromatography-Mass Spectrometry) analysis

The phytochemical investigation of methanolic extract was performed on GC-MS instrument (Thermo Scientific Co.). Experimental conditions of GC-MS system were as follows: TG-SQC column, dimension: 15 m, ID: 0.25 mm, Film thickness: 0.25 µm. The flow rate of mobile phase (carrier gas: Heli) was set at 0.8 ml/min. In the gas chromatography part, temperature program (oven temperature) was 50 °C raised to 250 °C at 5 °C/min and the injection volume was 10 µl. The results were compared by using the NIST Spectral library search program.

2.1.2 In vitro antacid activity of leaf extracts

Preparation of the extracts of various solvents

Dried leaves (1 kg) of *Ruellia tuberosa* L. was macerated with ethanol 96%, ethanol 70% ethanol 50%, and methanol for 48 h at room temperature, respectively. The extracts were filtered through a 45 µm filter. The resulting solution was concentrated in vacuum to dryness to give ethanol 96%, ethanol 70%, ethanol 50%, and methanol extracts. In addition, dried leaves were extracted by distilled water heated at 80 ^{o}C. The resulting solution was freeze-dried to get water extract.

All extraction was repeated for 3 times to get maximum extracts. These extracts of various solvents were used to study on *in vitro* antacid activity.

Preparation of artificial gastric acid

Two grams of salt and 3.2 mg of pepsin enzymes were dissolved in 500 mL water, 7.0 mL hydrochloric acid, and adequate water were added to make a 1000 mL solution of the artificial gastric acid at pH 1.20.

Control Drug Solution

- Sodium Bicarbonate (SB): 500 mg SB powder were weighed and dissolved and made up to 500 mL with distilled water.
- Edoz kids (EK) powder: 800 mg powder were weighed and dissolved and made up to 800 mL with distilled water.

Sample Solution

250 mg ethanol 96%, ethanol 70% and ethanol 50%, water, and methanol extracts were weighed and dissolved in 5 mL methanol and then made up to 250 mL with distilled water.

***In vitro* antacid activity assay**

The assay was conducted according to the method of Wu et al. (Wu T.H. *et al.*, 2010), and appropriately modified.

pH determination of the prescription decoction

One dose of prescription decoction (90 mL) was used for the pH determination at temperatures ranging from 25 ºC to 37 ºC. The pH values of the control solutions and SB were also determined for comparison.

Determination of the neutralizing effects on artificial gastric acids

90 mL of each test solution was added to 100 mL artificial gastric juices at pH 1.2. The pH values were determined to examine the neutralizing effect.

Determination of the neutralization capacity in vitro using the titration method of Fordtran's model

90 mL of the test sample was placed in a 250-mL beaker and warmed to 37 ºC. A magnetic stirrer was continuously stirred at 30 rpm to imitate the stomach movements. The test samples were titrated with artificial gastric juice to the endpoint of pH 3. The consumed volume (V) of artificial gastric juice was measured. The total consumed hydrogen ion (mmol) was measured as 0.063096 (mmol/mL) × V (mL).

Statistical analysis

Experimental data were expressed as mean ± SD. Comparisons between groups were analyzed by ANOVA test. The differences were considered to be statistically significant when $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Phytochemical constituents from methanolic extracts of Ruellia tuberosa L.

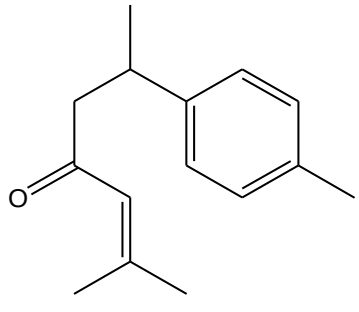
The GC-MS analysis showed 18 chemical compounds corresponding to 99.67% of total extract from *Ruellia tuberosa* L. leaves. Ar-turmerone (26.35%), β-Sitosterol (21.64%), Silanediol, dimethyl-,diacetate (9.18%), Curlone (7.59%), Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene- (7.54%) were the chief chemical compounds while 13 others compounds were present in minor quantities with peak areas ranging from 0.35–5.35%.

Table 1. Compounds identified in the methanol extract of Ruellia tuberosa L. by GC-MS

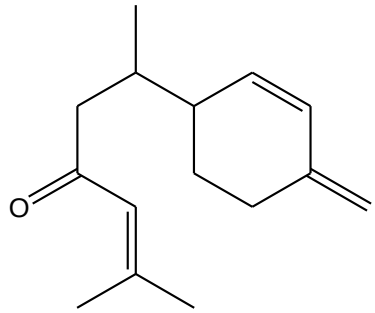
No	RT	Peak Area (%)	Molecular formula	Name of the compound
1	3.76	9.18	$C_6H_{12}O_4Si$	Silanediol, dimethyl-,diacetate
2	4.22	0.35	$C_3H_6O_3$	Methoxyacetic acid, 10-undecenyl ester
3	4.89	0.59	$C_{16}H_{12}O_2$	2-Pentanone, 4-hydroxy-4-methyl-
4	18.35	5.35	$C_{15}H_{22}$	Benzen, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-
5	18.49	4.67	$C_{15}H_{24}$	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [*S-(R*,S*)*]-
6	18.63	1.43	C_6H_{10}	Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, *(S)*-
7	18.79	7.54	$C_{15}H_{24}$	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [*S-(R*,S*)*]-

No	RT	Peak Area (%)	Molecular formula	Name of the compound
8	19.29	1.49	$C_{12}H_{18}O_2$	Bicyclo[3.1.1]hept-2-en-6-ol, 2,7,7-trimethyl-, acetate, [1*S*-(1α,5α,6β.)]-
9	19.38	0.51	$C_{13}H_{14}$	1-Naphthalenepropanol, α-ethyldecahydro-5-(hydroxymethyl)-α,58a-trimethyl-2-methylene-, [1*S*-[1-α-*S**
10	19.48	2.02	$C_{11}H_{16}$	7-Methoxymethyl-2,7-dimethylcyclohepta-1,3,5-triene
11	19.71	0.75	$C_{12}H_{20}O$	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, acetate, *(E,E)*-
12	19.95	26.35	$C_{15}H_{20}O$	Ar-turmerone
13	20.13	0.91	$C_7H_{12}O$	Cyclopentaneacetaldehyde, 2-formyl-3-methyl-α-methylene-
14	20.22	7.59	$C_{15}H_{22}O$	Curlone
15	20.42	4.67	$C_{15}H_{24}$	β-Guaiene
16	20.51	1.07	$C_6H_{10}O_2$	Cyclopentanecarboxylic acid, 3-isopropylidene, bornyl ester
17	20.7	3.57	$C_{15}H_{26}$	1H-Indene, 2,3,3a,4,7,7a-hexahydro-2,2,4,4,7,7-hexamethyl-
18	20.85	21.64	$C_{29}H_{50}O$	β-Sitosterol
19		0.33		No identified

Ar-turmerone and Curlone were identified as the main constituents of essential oils of turmeric (*Curcuma longa*) (Vijayastelter B.L. *et al.*, 2011). It was proven to reduce the gastric ulcer in rat stomach as seen from the ulcer index and histopathology of the stomach (Vijayastelter B.L. *et al.*, 2015).



Ar-tumerone



Curlone

Figure 1. Crucial constituents from leaf extract from Ruellia tuberosa L.

3.2 In vitro antacid activity of leaf extracts of Ruellia tuberosa L.

3.2.1 pH values of the tested solutions at temperatures ranging from 25 °C to 37 °C

The pH values of the MeOH and EtOH 96% extracts solutions at temperatures from 25 °C to 37 °C ranged from 5.57 –5.59. The pH values of water, EtOH 70%, and EtOH 50% extracts from 7.35 – 7.39; 7.41 – 7.45 and 7.32 – 7.37, respectively. The pH values of Edoz Kids and SB solutions at temperatures from 25 °C to 37 °C ranged from 6.33 – 6.41 and 8.61 - 8.63, respectively. Therefore, the results indicated that the temperature did not affect pH significantly (Figure 2).

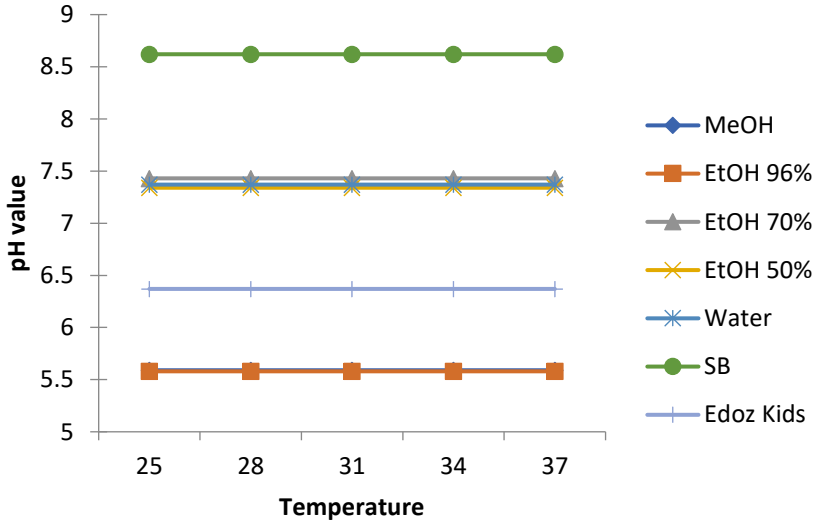


Figure 2. pH values of test samples determined at temperatures from 25 °C to 37 °C

3.2.2 Neutralizing effects on artificial gastric acids

When 90 mL of the test solution was added to 100 ml of the artificial gastric juice (pH 1.2), the pH values of MeOH, EtOH 96%, EtOH 70%, EtOH 50%, and Water extracts solutions were found to be 1.50 ± 0.015, 1.49 ± 0.006, 1.51 ± 0.015, 1.52 ± 0.006 and 1.50 ± 0.010, respectively. The pH values of Edoz Kids and SB solutions were 1.53 ± 0.010 and 1.57 ± 0.006, respectively. This result shows that the neutralizing capacity of SB was better than extracts, and Edoz Kids solution. The neutralizing capacity of EtOH 50% extract was stronger than the other ones and was not statistically significant difference that of Edoz Kids (Table 2).

Table 2. pH values as 90 mL of the test solution were added to 100 mL of artificial gastric acid juices

Samples	pH
MeOH extract	1.50 ± 0.015^{def}
EtOH 96% extract	1.49 ± 0.006^{g}
EtOH 70% extract	1.51 ± 0.015^{cde}
EtOH 50% extract	1.52 ± 0.006^{c}

Samples	pH
Water extract	1.50 ± 0.010^{efg}
SB	1.57 ± 0.006^{b}
Edoz Kids	1.53 ± 0.010^{c}

Data are presented as mean ± SD (n = 6). Values in the same column with the same letters are not significant difference (p>0.05). The letters are Grouping Information Using the Fisher LSD Method and 95% Confidence.

There have recently been surprising advances in the understanding of the pathophysiology and treatment of peptic ulcer disease (PUD). The ability of the gastric mucosa to resist injuries by endogenous secretions (acid, pepsin, and bile), and by ingested irritants (e.g. alcohol and NSAIDs) (Mertz H.R., Walsh J.H., 1991). It is generally accepted that it results from an imbalance between gastric aggressive factors and maintenance of the mucosal integrity through an endogenous defense mechanism (Holle G.E., 2010). The principal treatment of PUD includes antacids, H_2 receptor antagonists, and proton pump inhibitors. Among these, antacids have been widely used in the treatment of ulcers for many years. Antacids act by neutralizing gastric acid and help in the healing of ulcers, though, they do not decrease the volume of gastric secretion (Berstad A.,Weberg R., 1986).

Sodium bicarbonate (SB) shows the potential antacid activity. However, it should be avoided because it contains significant amounts of sodium and may alter the systemic pH. Besides, drug interactions related to SB have been frequently reported. Side effects and drug interactions are major clinical problems associated with antacid therapy (Sadowski D.C., 1994). Therefore, traditional herbal medicines have recently generated increasing interest in the treatment of ulcer disease. The 50% ethanol leaf extract of *Ruellia tuberosa* L. has the neutralizing activity that was not statistically significant difference when compared with a commercial product (Edoz Kids). It is expected that this was a potential antacid product but few side effects and drug interactions.

3.2.3 Physical antacid potency (neutralization capacity) in vitro

The consumed volumes (mL) of artificial gastric juices to titrate to pH 3.0 for MeOH, EtOH 96%, EtOH 70%, EtOH 50% and water extracts were 2.10 ± 0.050, 1.32 ± 0.029, 2.80 ± 0.050, 3.22 ± 0.029, 2.90 ± 0.050, respectively. The above results indicated that EtOH 50% was also the best neutralization capacity in other extracts. Similarly, the total consumed hydrogen ion at EtOH 50% extract was higher than others with 0.20 ± 0.002 mmol H^+ although it was 2.3 times lower than SB and 3.75 times lower than Edoz Kids. The results were showed clearly in Table 3.

Table 3. Consumed volume of artificial gastric juice and hydrogen ion (mmol) in the titration of 90mL test samples with pH 1.2 artificial gastric juice to the endpoint of pH 3

Sample	Consumed volume of artificial gastric juice (mL)	mmol H^+
MeOH extract	2.10 ± 0.050^{g}	0.13 ± 0.005^{g}
EtOH 96% extract	1.32 ± 0.029^{i}	0.08 ± 0.002^{i}

Sample	Consumed volume of artificial gastric juice (mL)	mmol H^+
EtOH 70% extract	2.80 ± 0.050^{f}	0.18 ± 0.003^{f}
EtOH 50% extract	3.22 ± 0.029^{d}	0.20 ± 0.002^{d}
Water extract	2.90 ± 0.050^{e}	0.18 ± 0.003^{e}
SB	7.34 ± 0.029^{c}	0.46 ± 0.003^{c}
Edoz Kids	11.90 ± 0.050^{b}	0.75 ± 0.005^{b}

Data are presented as mean ± SD (n = 6). Values in the same column with the same letters are not significant difference (p>0.05).The letters are Grouping Information Using the Fisher LSD Method and 95% Confidence.

The commercial control drugs such as SB and Edoz Kids, exhibited significant antacid potency. Simultaneously, neutralization capacities of EtOH 96% were lower than other extracts. While neutralization ones of EtOH 50% extract was the strongest in all extracts. Primarily, this result shows a similarity to antiulcer activity of 50% ethanolic leaf extract of *Ruellia tuberosa* L. in India (Manikandan A. and Victor A.D.D., 2009).

4. CONCLUSIONS

The methanol leaf extract of *Ruellia tuberosa* L. that analysed by GC-MS identified the 18 phytochemical constituents. In fact, ar-turmerone (26.35%), β-Sitosterol (21.64%), Silanediol, dimethyl-,diacetate (9.18%), Curlone (7.59%), Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene- (7.54%) were the chief chemical compounds. In addition, the 50% ethanolic leaf extract was the strongest neutralizing effects on *in vitro* antacid assay by an artificial gastric acid model. It is hoped that this would be a potential antacid product with few side effects and drug interactions in next time.

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