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Original Article

Anti-mitotic Activities of Ethanolic Extract and Glutinol from *Uvaria rufa* Blume

Agnes B. Alimboyoguen[1,*], Kathlia A. De Castro-Cruz[2], Chien-Chang Shen[3], Po-Wei Tsai[4,*]

[1]Research Center and Physical Science Department, College of Arts and Sciences, Cavite State University, Indang, Cavite, PHILIPPINES.
[2]School of Chemical, Biological and Materials Engineering, and Sciences, Mapúa University, Metro Manila, PHILIPPINES.
[3]National Research Institute of Chinese Medicine, Ministry of Health and Welfare, Taipei, TAIWAN.
[4]Department of Medical Sciences Industry, Chang Jung Christian University, Tainan, TAIWAN.

ABSTRACT

Background: This study describes the isolation of major chemical constituent from the bark of *Uvaria rufa* Blume, commonly known as "allagat or susong kalabaw", an indigenous Philippine medicinal plant that belongs to the family *Annonaceae*. It is a short climbing shrub that grows in low and medium-altitude forests in the country. **Materials and Methods:** The bark samples of *U. rufa* were air-dried for three months without exposure to sunlight. The samples were ground in a blender, then, soaked in CH_2Cl_2 and in ethanol for three days and then, filtered. The crude extract of *U. rufa* was chromatographed on a gravity column dry packed with silica gel and was fractionated by silica gel chromatography using increasing proportion of acetone in CH_2Cl_2 at 10% increment. The purified isolates were subjected to NMR for structure elucidation. Their structures were identified mainly by using ^{1}H-NMR and by comparing current NMR data with those reported in the literature. **Results:** The results showed that the air-dried bark dichloromethane extract of the plant afforded glutinol. Glutinol and ethanolic extract exhibited anti-mitotic activity (18.33 % and 18.93 %, respectively), comparable to methotrexate (19.07 %), a well-known anti-cancer drug and even more potent in which they surpassed by 0.74 % and 0.14 %. **Conclusion:** The dichloromethane extract of the bark of *U. rufa* afforded glutinol that can be one the main metabolites responsible for the anti-mitotic activity of the plant. It may also contained other substances that might have great potential as novel therapeutic agents for cancer.

Keywords: *Uvaria rufa*, Allagat, Glutinol, Anti-mitotic, Triterpene.

Correspondence:
Dr. Agnes B Alimboyoguen
Research Center and Physical Science Department, College of Arts and Sciences, Cavite State University, Indang, Cavite 4122, PHILIPPINES.
Email id: alim.agnes.b@gmail.com

Dr. Po-Wei Tsai
Department of Medical Sciences Industry, Chang Jung Christian University, Tainan 711, TAIWAN.
Email id: powei@mail.cjcu.edu.tw

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INTRODUCTION

Uvaria rufa Blume (*U. rufa*), commonly known as "allagat or susong kalabaw", is an indigenous Philippine medicinal plant that belongs to the family *Annonaceae*. It is a short climbing shrub that grows in low and medium-altitude forests in the country. Traditionally, all parts of this plant are used in treating several ailments. Infusion of leaves is used for treating fever while decoction of dried stem is commonly employed in treating haemorrhage, skin allergies,[1,2] intestinal ulcers and prostate disorders including BPH.[3] In addition, the root extract can also induce urine contractions (ecbolic) in pregnant women and the fruits which have sweet flavour are edible and can cure intestinal ulcers.[4] Literature search also revealed that both ethanol and ethyl acetate extracts of *U. rufa* leaves found to have antioxidant properties,[5] while chloroform extract and fractions exhibited antituberculosis activity.[6] Reports also indicated the antibacterial,[7] and antispasmodic properties of ethanol extract of twigs and stem of the plant.[8]

Several studies have been reported on their chemical constituents and some biological activities. Flavonoid glycosides such as kaempferol 3-O-ß-galactopyranoside, rutin, astragalin, isoquercitrin-6-acetate and isoquercitrin, isolated from the leaves of *U. rufa* inhibited the formation of advanced glycation end-products (AGEs) which contribute to the progression of diabetic complications and aging in the bovine serum (BSA) albumin-glucose assay.[9] Another study also showed the *in vitro* inhibitory activity of the flavonoids,[10] and terpenoids,[6] against Mycobacterium tuberculosis. Lignan glycoside, ufaside along with the compounds, oxoanolobine and ergosta-4, 6, 8,[11] 22-tetraen-3-one found to be cytotoxic against the human lung adenocarcinoma.[12] The presence of alkaloids such as liriodenine, lanuginosine, oxoanolobine, roemerine, anonaine, xylopine and roemeroline indicated significant antioxidant properties.[13] Literature study also reported that the leaf oil of *U. rufa* were composed of δ-3-carene, *n*-hexadecanoic acid, β- caryophyllene, (*Z*)-β-ocimene, γ-terpinene and humulene,[14] while the stem oil

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was dominated by germacrene, benzyl benzoate and *n*-eicosane.[11] Four new polyoxygenated cyclohexene derivatives, uvarirufone and uvarirufols, along with ten related known compounds, were also isolated from the EtOH extract of the aerial parts of *U. rufa*.[15]

In this study, we report here the isolation, structure elucidation and anti-mitotic activity of glutinol (Figure 1), a pentacyclic triterpene, using the *Allium cepa* assay. The anti-mitotic activity of the crude dichloromethane and ethanolic extracts of *U. rufa* were also evaluated and compared with glutinol obtained from the dichloromethane extract of the plant. Although, glutinol was already isolated from the plant, to the best of our knowledge, this is the first report on the anti-mitotic activities of this triterpene and crude extracts from *U. rufa*.

MATERIALS AND METHODS

Sample Collection and Identification of *U. rufa*

The bark samples (A voucher specimen with No. CvSU-UR-1) of *U. rufa* were collected at Sinait, Ilocos Sur of the Philippines in December, 2018. The samples were identified as *U. rufa* at the Jose Vera Santos Herbarium, Institute of Biology, University of the Philippines, Diliman, Quezon City, Philippines.

Sample Preparation and Extraction

The bark samples of *U. rufa* were air-dried for three months without exposure to sunlight. The samples were ground in a blender, then, 0.83kg was soaked in CH_2Cl_2 and the remaining 0.2 kg in ethanol for three days and then, filtered. Each filtrate was concentrated under vacuum to afford crude CH_2Cl_2 and ethanol extracts of 36.9 g and 1.91g, respectively.

Isolation and Purification of Glutinol

The crude extract of *U. rufa* was chromatographed on a gravity column dry packed with silica gel (100-280 mesh) and was fractionated by silica gel chromatography using increasing proportion of acetone in CH_2Cl_2 at 10% increment. Eleven fractions were collected. The 40% - 60% acetone in CH_2Cl_2 fraction were combined and rechromatographed (2x) using CH_2Cl_2 followed by 15% ethyl acetate in petroleum ether (11x) to afford AB43 (9 mg). Fractions were monitored by TLC which was performed with plastic-backed plate coated with silica gel F_{254}. The spots were visualized by spraying with vanillin-H_2SO_4 followed by warming.

Structure Elucidation of Glutinol

All TLC pure isolates were sent to National Research Institute of Chinese Medicine, Taiwan for NMR analyses. The NMR were recorded on a Varian VNMRS Spectrometer in $CDCl_3$ at 600 MHz for ^{1}H- NMR and 150 MHz for ^{13}C-NMR spectrum.

Antimitotic Activity

Antimitotic activity was evaluated by using the meristematic cells of *A. cepa* roots. *A. cepa* bulbs were sprouted in tap water at room temperature, when the roots were about 5 mm long the bulbs were placed on beakers containing the test samples (10 mg/ml) such that the roots were immersed in the samples. The sprouted roots were also treated with 1% DMSO (Control group) and methotrexate (0.1 mg/ml, Standard drug). One hour later the root tips were cut and transferred to fixing solution of 45 % acetic acid and 95 % ethanol in the ratio of 1:3 v/v (10-12 hr) followed by warming the root tips in ^{1}N HCl in oven at 50°C for 15 min and then stained with Farmer's solution. The slide was observed under microscope to record the number of non-dividing and dividing cells mitotic index.

RESULTS AND DISCUSSION

^{1}H-NMR, ^{13}C-NMR, HSQC, COSY and HMBC of Glutinol

The glutinol from CH_2Cl_2 extract of the air-dried bark of *U. rufa* was obtained as white solid. The compound produced a blue-violet TLC spot when warmed with vanillin-sulfuric acid visualizing agent. It has an R_f value of 0.54 when developed in 20% % EtOAc in petroleum ether. Its structure was elucidated by extensive 1D and 2D NMR spectroscopy as described below.

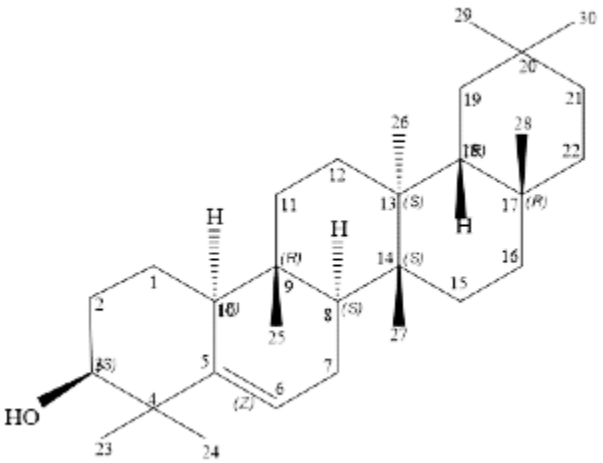

Figure 1: Glutinol from the bark of *U. rufa*.

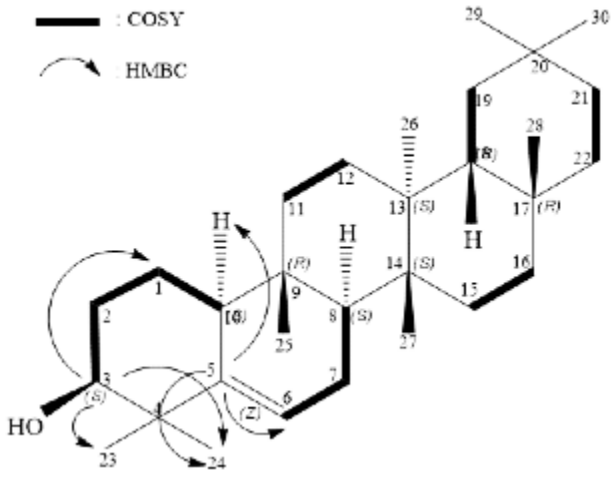

Figure 2: ^{1}H–^{1}H COSY and key 1 H–13C long-range correlations of Glutinol.

The ^{1}H NMR spectrum of glutinol (Table 1) gave resonances for olefinic proton at δ 5.62 (d, J= 6.0 Hz), an oxymethine proton at δ 3.46 (d, J= 7.2 Hz) and eight methyl singlets at δ 0.83, 0.93, 0.96, 0.98, 1.02, 1.07, 1.12 and δ 1.15. The shielded region of the spectrum indicated overlapping resonances for methylene and methine protons which are typical of triterpenes. The ^{13}C NMR spectrum of the compound (Table 1) indicated signals for thirty carbons. An oxymethine carbon at δ 76.7 and two

Table 1: The Complete Spectral Data of Glutinol.

Position	^{13}C Chemical Shift, δ_C (ppm)	Correlated Proton Chemical Shift, δ_H (ppm)	HMBC Correlations
1	18.2	1.48, 1.54	1.85, 2.01
2	27.8	1.69, 1.85	1.54, 1.48
3	76.7	3.46 (d, J= 7.2 Hz)	1.12, 1.02, 1.48
4	40.8	-----	5.6, 1.02, 1.12
5	141.6	-----	2.01, 1.84, 1.12, 1.02, 1.47,148
6	122.1	5.62 (d, J= 6.0 Hz)	2.01, 1.84, 1.97
7	23.6	1.84, 1.97	5.60, 1.51
8	47.4	1.51	2.01, 5.60, 1.97, 1.84, 1.54, 0.83
9	34.8	-----	1.51, 0.83, 1.54, 1.39, 2.01, 1.48, 1.35
10	49.7	2.01	1.54, 0.83, 1.39, 5.60, 1.51, 1.48
11	34.6	1.39, 1.54	1.51, 1.39, 2.01, 0.83
12	30.3	1.35, 1.35	1.07, 1.54, 1.39
13	39.3	-----	1.07, 1.25, 1.39, 0.98, 1.60, 1.35
14	37.8	-----	1.07, 0.98, 1.51, 1.30, 1.47, 1.39, 1.54
15	32.1	1.30, 1.47	0.98, 1.54, 1.39
16	36.0	1.39, 1.54	1.15, 1.30, 1.47
17	30.1	------	1.15, 1.54, 1.39, 1.25, 1.47, 1.60, 0.92
18	43.0	1.60	1.07, 1.54, 1.25, 1.39, 0.92, 1.35, 1.51, 1.47
19	35.1	1.25, 1.39	1.60
20	28.2	-----	0.93, 0.96, 1.25, 0.92, 1.54,1.60, 1.47, 1.39
21	33.1	1.25, 1.47	0.93, 0.96, 1.25, 1.39, 0.92, 1.54
22	38.9	0.92, 1.54	1.15, 1.25, 1.47, 1.39, 1.54, 1.60
23	25.4	1.12 (s)	1.02
24	28.9	1.02 (s)	1.12
25	16.2	0.83 (s)	2.01, 1.51, 1.39
26	19.6	0.98 (s)	------
27	18.4	1.07 (s)	1.30
28	32.0	1.15 (s)	0.92, 1.54, 1.39, 1.60
29	34.5	0.93 (s)	0.96, 1.25, 1.47, 1.39
30	32.4	0.96 (s)	0.93, 1.39, 1.25, 1.47

Table 2: Total number of cells, dividing cells and mitotic index of glutinol and crude dichloromethane and ethanolic extracts of *U. rufa*.

Test Sample	Total Number of Cells	Number of Dividing Cells	Mitotic Index
Control (1% DMSO)	909	691	76.18
Control (Methotrexate)	856	163	19.07
Glutinol	924	174	18.93
Ethanolic Extract	858	156	18.33
Dichloromethane Extract	907	282	30.91

olefinic carbons, a non-protonated at δ 141.6 and a protonated at δ 122.1 were detected. And, the remaining twenty-eight carbon resonances were attributed to methyl, methylene, methine and quaternary carbons. The COSY spectrum gave six isolated spin systems as follows: H-10/H_2-1/H_2-2/H-3; H-6/H_2-7/H-8; H_2-11/H_2-12; H_2-15/H_2-16; H-18/H_2-19; H_2-21/H_2-22 (Figure 1). The ^1H and ^{13}C NMR connectivity's in 1 (Table 1) were verified by HSQC and its structure was elucidated by analysis of the HMBC 2D NMR data with key HMBC correlations shown in Figure 2.

The hydroxyl was attached to C-3 since long-range correlations were observed between H_2-2, H_3-23, H_3-24 and C-3. This attachment of hydroxyl to C-3 can be also attributed from the peak resonance observed at δ 3.62 and may cause intramolecular H-bonding which prevented the proton exchange and resulted in the proton detection by the NMR. The non-protonated olefin was assigned to C-5 and was connected to the doubly bonded protonated olefin C_6 due to the correlations of this non-protonated olefinic carbon to the non-equivalent methylene protons H-7, an olefinic proton H-6, a methine H-10 and 2 methyl protons H-23 and H-24. All long-range correlations are consistent with the structure of glutinol as reported from the literature.[4,16] The compound has a molecular formula of $C_{30}H_{50}O$ and its structure indicated the presence of an alkene and alcohol functionality.

This compound was found to have analgesic property,[17] and anti-inflammatory activity which may be useful as template for the development of new anti-inflammatory drugs.[18]

Anti-mitotic Activity of Extracts of *U. rufa* and Glutinol

As part of our continuing search for bioactive compounds from Philippine medicinal plants, glutinol and crude dichloromethane and ethanolic extracts of *U. rufa* were tested for anti-mitotic activity using *Allium cepa* assay. As shown in Table 2, the mitotic indices of all test samples significantly reduced after 3 hr treatment when compared with the control (1% DMSO). At the same time, glutinol and ethanolic extract exhibited anti-mitotic activity comparable to methotrexate, a well-known anti-cancer drug. The ethanolic extract and glutinol, isolated from the dichloromethane extract of *U. rufa* obtained mitotic index of 18.33% and 18.93 %, respectively, even more potent in which they surpassed the anti-mitotic potential of methotrexate (19.07 %) by 0.74 and 0.14%.

The reduction of mitotic index or number of dividing cells suggested that *U. rufa* contains polar and non-polar substances other than glutinol that may inhibit mitosis or cell division. These substances present in both ethanol and dichloromethane extracts of *U. rufa* can be potential antimitotic drugs that are efficient in the inhibition of growth of cancer cells. Antimitotic drugs are substances that inhibit polymerization dynamics of microtubules which are formed during interphase and important for chromosome segregation and cell division undergoing mitosis.[19]

CONCLUSION

The dichloromethane extract of the bark of *U. rufa* afforded glutinol that can be one the main metabolites responsible for the anti-mitotic activity of the plant. It may also contain other substances that might have great potential as novel therapeutic agents for cancer. However, it is suggested that further study and isolation of metabolites be conducted as well as the mechanism of action of plant material to understand the molecular pathways needed in designing synergistic treatments for much more effective in fighting different types of cancer.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

***U. rufa*:** *Uvaria rufa*; ***A. cepa*:** *Allium cepa;* **NMR:** Nuclear Magnetic Resonance; **TLC:** Thin Layer Chromatography; **R_f value:** Retention Factor value; **EtOAc:** Ethyl Acetate; **DCM:** Dichloromethane.

SUMMARY

This study describes the isolation of major chemical constituent from the bark of *Uvaria rufa* Blume, commonly known as "allagat or susong kalabaw", is an indigenous Philippine medicinal plant that belongs to the family *Annonaceae*. The results showed that the air-dried bark dichloromethane extract of the plant afforded glutinol. Glutinol and ethanolic extract exhibited anti-mitotic activity (18.33% and 18.93%, respectively), comparable to methotrexate (19.07%). It may also contain other substances that might have great potential as novel therapeutic agents for cancer.

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