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Original Research Article

Evaluation of the Phytochemical Constituents, Toxicity Profile, and Pharmacological Activities of Qust al-Hindi Herb (Saussurea costus (Falc.))

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ABSTRACT

Qust al-Hindi or *Saussurea costus* (Falc.) Lipsch. was first introduced as a supplement therapy. The herb is widely circulated in various markets in Indonesia and several other countries to treat respiratory tract disorders, including cough and bronchial asthma, and to support treatment of viral infections such as SARS-CO2. Hence, it is necessary to determine its toxicity profile, pharmacological properties, and phytochemical constituents. This research aims to evaluate the pharmacological activities of Qust al-Hindi extract, including the immunomodulatory and anti-inflammatory in animal models, as well as the antioxidant activity, cytotoxicity, and acute toxicity. The chemical compounds were determined by Gas Chromatography-Mass Spectroscopy (GC-MS). Extraction was carried out with ethanol, followed by phytochemical screening and fractionation with hexane, ethyl acetate, and ethanol for metabolite identification by GC-MS. Qust al-Hindi extract exhibited cytotoxicity in BSLT and MTT assays and showed significant antioxidant, immunomodulatory, and anti-inflammatory activities. Its cytotoxic activity was shown to be active against both BSLT and A549 cell lines. The 1,3-cyclooctadiene, dehydrocostus lactone, 4H-Pyran-4-one, and 2,3-dihydro-3,5-dihydroxy-6-methyl compounds have proved to provide potential pharmacological activities and were predominantly present in the hexane, ethyl acetate, and ethanol fractions, respectively. The research revealed that the Qust al-Hindi herbal product presents beneficial pharmacological activities that need further exploration.

Keywords: Herbal; pharmacological assays; Qust al-Hindi; *Saussurea costus*; Supplement therapy

Introduction

Consumer interest in natural herbal products is increasing in many developing countries.[1-2] These herbal products are increasingly being developed into medicinal products, nutraceuticals, and cosmetics.[3] *Saussurea costus* (Falc.) Lipsch known as Qust al-Hindi is an ancient herb from 2500 years ago, known and used in various ancient systems of medicine.[4] The herb is widely used as a traditional medicine in many countries worldwide. In the Indian traditional medicine, it is used either alone or in combination with other drugs.[5] This herb is frequently used for inflammatory diseases in the traditional Korean prescriptions.[6] Since its extensive traditional knowledge is based on indigenous health care techniques, this herb is widely employed in the Himalayan traditional medicine systems.[7] The benefits of this plant are also widely recognized in the prophetic medicine of Ayurvedic, Chinese, and Tibetan.[8] In addition, various studies have shown that this plant is active as an anti-ischemic in heart disease, hypoglycaemic, antimicrobial, antiparasitic, and anticonvulsant agent.[5,9-11]

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Saussurea costus is a plant of medicinal importance and is rich in pharmacologically active compounds. Various compounds have been identified, including sesquiterpene terpenes, anthraquinones, alkaloids, and flavonoids. The sesquiterpene terpenes are the major constituents of the root of *S. costus* and have been reported to possess medicinal properties and a variety of bioactivities.[12]

As a traditional medicine and supplement product, Qust al-Hindi is widely traded in Indonesia and has unexpectedly become one of the essential traditional medicines in Indonesia to support various types of therapy, including as an antihypertensive and antidiabetic as well as for pneumonia and liver disorders. In addition, Qust al-Hindi herbal product was widely circulated amidst the COVID-19 era among the public as it can be used to treat fever, nausea, cough, and bronchial asthma. In addition, its chemical compounds have been shown to act as a bronchodilator, and its hexanoic acid functions as an expectorant.[13-14] A previous study reported that there was a reduction in the number of patients treated for a particular disease with the use of these supplements although not at a significant level if the variable regulation was enforced.[15]

Due to the massive use of this herb as a supplementary therapy for a variety of treatments, while knowledge of the appropriateness of indications in patients remains limited, further testing is needed regarding their pharmacological activities. Hence, in this study, the phytochemical and pharmacological activities of the Qust al-Hindi herb supplement were tested according to its activities as complementary therapy of immunomodulatory, anti-inflammatory, and antioxidant, as well as its cytotoxic activity.

Materials and Methods

Ethical Approval

The use of experimental animals in this study was approved by the Health Research Ethics Committee of the Faculty of Medicine of Universitas Indonesia - Dr. Cipto Mangunkusumo Hospital (Nr. KET-720/ UN2.Ft/ ETIK/ PPM.00.02/2021). Female Swiss albino mice were used in the anti-inflammatory assays and female Sprague-Dawley rats in the immunomodulatory and toxicity assays. The use of single-sex (female) experimental animals refers to the OECD guidelines.[16]

Materials

The Qust al-Hindi Powder Product (QHPP) was obtained from a traditional medicine supplier in South-Jakarta, Indonesia (6.25°S 106.8°E) purchased in September 2021. The *Artemia salina* larvae were obtained from Golden West Artemia. The A549 cells (ATCC CCL 185), DMEM, 5% foetal bovine serum (FBS), l00 U/mL penicillin and 100 µg/mL streptomycin were obtained from Primate Animal Studies Center, Bogor Agricultural Institute. The standard Doxorubicin and vitamin C were purchased from Merck while the isoprinosine syrup was purchased from Darya Varia. The sheep red blood cells (SRBC) isolated from fresh blood and obtained from Hospital and Laboratory Equipment and General Trade PT Gajah Mungkur Indotama were used as antigens. The 2.2-diphenylpicrylhydrazyl (DPPH), Turk's solution, EDTA, Phosphate Buffer Saline, Na-CMC, and carrageenan were purchased from Sigma Aldrich. All the chemical reagents used were of analytical grade.

Extraction and Fractionation

One kg of QHPP was macerated with 15 L ethanol for three days with three replications. The extract was then concentrated using a rotary evaporator at 50ºC to obtain a dried crude extract. The crude extract was then fractionated successively with 5 L of hexane, 5 L of ethyl acetate, and 4 L of ethanol. All the fractions were subjected to GC-MS analysis.

Physicochemical Evaluation

The crude extract was analysed for its physicochemical parameters of moisture and ash content in accordance with the standard guidelines of the Indonesian Herbal Pharmacopoeia and WHO guidelines.[17-18]

Phytochemical Screening

The crude extract was qualitatively identified to determine the presence of alkaloid, phenol, flavonoid, tannin, steroid, triterpenoid, and saponin. The procedures were carried out in accordance with the Herbal Pharmacopoeia of Indonesia (2017) and Tiwari *et al*. (2016).[19-20]

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The analysis of the volatile components in each fraction was performed on an Agilent 6890N GC-MS coupled to an Agilent 5973 MSD. The oven temperature was set at 250°C, and the injection temperature was 280°C. The carrier gas used was He (helium) at a flow rate of 1 ml/min. The detector was operated in EI mode (70 eV) for three replicates. The ion source temperature was 230ºC and the scanning range of 40-500 m/z. The ion fragmentation formed was detected with an analyser based on the mass ratio.[21]

Acute Toxicity Assay

The toxicity of QHPP extract was evaluated with an acute toxicity test. The determination was conducted according to the OECD guidelines.[16] All the female Sprague-Dawley rats were initially given the extract at a single dose of 2000 mg/kg BW to determine the effect on mortality. If there were no deaths, the 2000 mg/kg BW dose was repeated. The tests were performed in an average of 2-4 stages to obtain an assessment of the acute toxicity of the extract, depending on the number of animal deaths. Each phase was conducted for 14 days after treatment, and then the number of animal deaths, signs of toxicity, changes in body weight, and macroscopic and organ weights were observed. The weights of the test animals' organs, including the liver, kidney, heart and ovary, were analysed and compared with those of the normal group.

Immunomodulator Assay with Antibody Titter Method

The immunomodulatory effect of the extract was determined by the antibody titer test.[22] On day 1, all the female Swiss albino mice were immunized with 1×10^8 cells/ml SRBC intraperitoneally. The raw extract was administered orally to the test group at doses of 100, 200 and 400 mg/kg BW once daily for 7 days. Isoprinosine at 10.27 mg/kg BW was administered orally to the positive control group. Blood samples were collected from the mice on day 8 by retro-orbital bleeding and centrifuged to obtain blood serum. The blood serum was inactivated at 56ºC for 30 minutes in a total of 25µL from each group of mice. The inactivated serum solution was then put into a well plate containing 25 µL of Phosphate Buffer Saline (PBS). Then, the solution was diluted in stages into the corresponding wells (1:2; 1:4; 1:8; 1:16; 1:32; 1:64; 1:128; 1:256; 1:512; 1:1024), added with 25 µL of 2% SRBC, and stored at 25°C for two hours.[22] The determination was executed by observing the hemagglutination carried out in a 96-well (12x8) microplate. The reaction resulted in agglutination. To convert the geometric series obtained by reading the titer into an arithmetic series, the following formula was used.[23]

Anti-Inflammatory Assay

The anti-inflammatory activity of QHPP extract was tested by using the carrageenan induction method. A total of 36 rats were used in 6 groups, in accordance with the General Methods for Research and Assessment of Herbal Medicinal Products.[17] The control and positive groups were administered with 0.5% Na-CMC solution and diclofenac sodium (10.28 mg/kg BW), and the three test groups were given extracts in a series of doses of 25, 75, and 150 mg/kg BW. All the doses were administered orally one hour before the administration of 1% carrageenan injection. The volume of rat foot edemawas measured using a Mercury Plethysmometer every 1, 2, 3, 4, and 5 hours. The percentages of edemaand inhibition were calculated.

Cytotoxicity Assays

Brine Shrimp Lethality Test (BSLT) Method

The Brine Shrimp Lethality Test (BSLT) method was carried out according to the method performed by Meyer et al. (1982). The primary solution of the extract was pipetted into five Eppendorf tubes, and each tube was added with ten 48-hour-old *Artemia salina* larvae pipetted from the incubator. Then, the solution was diluted with seawater to the limit of 1.5 mL to obtain concentration variations of 1000, 500, 250, 125, and 62.5 ppm. Each concentration variation of the test solution and control solution was prepared in three repetitions. The toxicity characteristics were analysed by calculating the % mortality of *Artemia salina* larvae at each concentration after 24 hours.[24]

Cytotoxic Activity on A549 Cells

The cytotoxicity test was carried out using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MMT) method.[25] The cells used in this study were A549 cells derived from human lung cancer cells cultured in Dulbecco's Modified Eagle Medium (DMEM) and inoculated into wells with 100 µL inoculants (5×10^3 cells/well density) for 24 hours. A total of 100 µL of the extract with various concentrations (7.8, 15.6, 31.2, 62.4, 124.8, 249.6, and 499.2 ppm) were added to the inoculant and incubated in a CO_2 incubator at 37ºC for 24 hours. The absorbance was read using a microplate reader (The APW-100 Microplate Washer) at 570 nm. The percentage of inhibition of A549 cells was calculated. The IC_{50} was determined using the curve of the relationship between the extract concentration (x) and the percentage of inhibition of A549 cells (y).

Analysis of Antioxidant Activity

The antioxidant activity of QHPP extract was confirmed with a method performed by Molyneux. Each crude extract was diluted with methanol to prepare the extract concentrations of 100, 150, 200, 250, and 300 ppm and mixed with DPPH solution. The absorbance of the sample was measured at the maximum wavelength after 30 minutes.[26] The IC_{50} was

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calculated using a regression equation, then the AAI (Antioxidant Activity Index) was calculated by dividing the DPPH concentration by the IC_{50} obtained.

Results and Discussion

Qust al-Hindi herbal products are widely circulated in Indonesia and commonly used by the public to support the treatment of COVID-19 patients. The present work investigated the Qust al-Hindi herbal product for several biological and pharmacological activities. The yield of QHPP ethanol extract obtained in the present research was 4.53%. Besides, the ethanol extract was partitioned further with hexane, ethyl acetate, and ethanol, and the yields obtained were 20.5%, 27.86%, and 9.04%, respectively.

The chemical compounds present in the raw materials and the manufacturing process affect the pharmacological activities of botanical products. Ensuring physicochemical properties is critical for consistent pharmacological activities and patient efficacy. The crude extract was evaluated for the physicochemical characteristics, and the results showed that the moisture content and ash content of QHPP extracts reached 9.26% and 7.9%, respectively, thus complying with the requirements of the Indonesian Herbal Pharmacopoeia and WHO guidelines.[18,26] The determination of the moisture content and ash content parameters of the extracts aimed to identify the quality of the extracts tested in this study.[25] Moisture content affects the stability of the extract in storage against fungal and microbial attacks, and water affects various chemical reactions that may occur.[18] Furthermore, screening of the chemical constituents of Qust al-Hindi herbal products proved that the chemical compounds include phenols, flavonoids, tannins, alkaloids, and triterpenoids. A previous study showed that terpenoids and flavonoids are the compounds responsible for various pharmacological activities of *Saussurea costus*.[11]

Moreover, the identification of the active components of Qust al-Hindi herbal products with GC-MS has revealed a correlation with the phytochemical constituents of the native plant. The 1,3-cyclooctadiene found in the hexane fraction examined in previous research by Jaradat et al. (2017) showed that the compound exhibited antioxidant and antibacterial activity.[27] Dehydrocostus lactone is a natural sesquiterpene lactone that has been reported as the primary and biologically active compound from the roots of *Saussurea costus* with various biological activities, one of which is as an antioxidant.[28-29] In addition, in the ethanol fraction was found 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl contained strong antioxidants.[8] The MS spectrum for each fraction can be seen in Tables 1-3.

Table 1: GC-MS library data of n-Hexane Fraction

R.Time	% Area	Name of Compound
7.824	0.07	(3E)-4-(2,6,6-Trimethyl-2- cyclohexen-1-yl)-3-buten-2-one
7.932	0.07	5,9-Undecadien-2-one, 6,10-dimethyl
8.209	0.10	3E)-4-(2,6,6-Trimethyl-1- cyclohexen-1-yl)-3-buten-2-one
8.629	0.15	2-(3-Isopropenyl-4-methyl-4-vinylcyclohexyl-2-propanol
8.902	0.27	Bicyclo(7.2.0)undec-3-en-5-ol,4,11,11 trimethyl -8-methylene-,[1R-(1R*,3E*,5R*,9S*)]
9.247	3.94	1,3-Cyclooctadiene
9.381	0.39	Caryophyllene-(I1)
9.493	0.36	2-Propenal, 3-(2,4,5,6,7,7a-hexahydro-3,7-dimethyl-1h-inden-4-yl)-2-methyl-, (4s-(4alpha(E),7beta,7aalpha))
9.577	0.43	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl
9.950	4.10	(Z,E)-7-Methyl-4-(1-methylethylidene)-1,7-cyclodecadienemethanol
10.089	0.42	1H-3a,7-Methanoazulene, octahydro-1,4,9,9-tetramethyl-
10.217	0.32	3,7-Dimethyl-3,6-octadien-1-ol
10.599	3.10	Pentadecanoic acid, 14-methyl-, methyl ester
11.451	2.63	Methyl 10-trans,12-cis-octadecadienoate
11.672	3.43	9,12-Octadecadienoic acid (Z,Z)-
11.861	0.40	13-Tetradece-11-yn-1-ol
14.158	0.40	7-Pentadecyne
14.643	0.08	Pregnenolone

Table 21: GC-MS library data of Ethyl Acetate Fraction

R.Time	% Area	Name of Compound
9.291	1.30	1H-Cycloprop[e]azulene, decahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.beta.,4a.beta.,7.beta.,7a.beta.,7b.alpha.)]
9.953	1.05	Valerenol
10.416	0.86	2 (3H)-Benzofuranone, 6-ethenylhexahydro-6-methyl-3-methylene-7-(1-methylethenyl)-, [3aS-(3a.alpha.,6.alpha.,7.beta.,7a.beta.)]
10.910	1.26	6,10-Dimethyl-3-methylene-3a,4,5,8,9,11a-hexahydrocyclodeca[b]furan-2(3h)-one
11.006	2.85	Germacra-1(10),4,11(13)-trien-12-oic acid, 6.alpha.-hydroxy-, .gamma.-lactone, (E,E)
11.031	2.20	Germacra-1(10),4,11(13)-trien-12-oic acid, 6.alpha.-hydroxy-, .gamma.-lactone, (E,E)
11.090	2.63	Germacra-1(10),4,11(13)-trien-12-oic acid, 6.alpha.-hydroxy-, .gamma.-lactone, (E,E)
12.153	5.05	(+)-Dehydrocostus lactone

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Table 32: GC-MS library data of Ethanol Fraction

R.Time	% Area	Name of Compound
5.701	10.17	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl
6.328	6.87	2-Furancarboxaldehyde, 5-(hydroxymethyl)

The toxicity of an extract is tested to determine the initial adverse effects of administering the extract as a remedy. The acute toxicity assays showed that no deaths were found in the experimental animals. In this research, the mice were given a single dose of 2000 mg/kg BW for 14 days. Based on the classification of the Globally Harmonized System (GHS), the test dose was included in category 5/unclassified.[30] It was observed that there was no difference in the signs of toxicity between the group receiving 2000 mg/kg BW and the control group. Organ weight is an important indicator of the physiological and pathological condition of an animal. The relative weight of organs is crucial in diagnosing injuries.[31] The results obtained from the relative weight of the organs can be seen in Table 4. The results showed no significant differences in all the animal organs ($p > 0.05$). The test showed that the crude extract did not affect the organ weights of the experimental animals. An increase in organ weight is an indication of hypertrophy while a decrease is an indication of necrosis of the target organs.[32] An increase in liver weight may indicate hepatocellular hypertrophy due to enzyme induction. Additionally, an increase in heart weight may indicate myocardial hypertrophy.[33] On the other hand, an increase in kidney weight may indicate nephrotoxicity.[34] Internal organs are crucial for metabolism. Through metabolic reactions, toxins affect the heart, liver, kidneys, and lungs.[17]

Table 4: Organ Relative Weight Measurements after QHPP Extract Administration

Groups	Mean of Organ Weight (g) ± SD				
	Liver	Heart	Lungs	Kidneys	Ovaries
C	6.34 ± 0.75	0.47 ± 0.04	1.05 ± 0.13	0.65 ± 0.00	0.03 ± 0.01
1	5.88 ± 0.71	0.44 ± 0.09	1.40 ± 0.12	0.56 ± 0.06	0.03 ± 0.01
2	7.13 ± 0.36	0.48 ± 0.04	1.75 ± 0.34	0.57 ± 0.07	0.03 ± 0.00
3	6.63 ± 0.98	0.43 ± 0.02	1.55 ± 0.21	0.63 ± 0.06	0.02 ± 0.00

*Comparison of the organ data of the control group at a dose of 2000 mg/kgBW was significantly different; C: Control, 1: repetition 1, 2: repetition 2, 3: repetition 3

Table 5: Antibody Titer Value (µL)

Groups	Treatments	Average of Antibody titer ± SD
NR	Induction of PBS + Na CMC 0.5% per oral	1.120 ± 0.269
NT	Induction of SRBC 2% + Na CMC 0.5% per oral	1.241 ± 0.330
R	Induction of SRBC 2% + Isoprinosine per oral	2.927 ± 0.785*+
D1	Induction of SRBC 2% + EEQH 100 mg/KgBW	2.204 ± 0.602*
D2	Induction of SRBC 2% + EEQH 200 mg/KgBW	2.084 ± 0.785
D3	Induction of SRBC 2% + EEQH 400 mg/kgBW	1.843 ± 1.174

* Significantly different from normal control ($p \leq 0.05$)
+ Significantly different from negative control ($p \leq 0.05$)
NR: Normal, NT: Negative, R: Reference, D1: Dose 1 (100 mg/kgBW), D2: Dose 2 (200 mg/kgBW), D3: Dose 3 (400 mg/kgBW)

To confirm the humoral response to SRBC, the hemagglutination of the antibody titter is determined. The results of the determination of immunomodulatory activity using the antibody titer test are shown in Table 2. The Wilcoxon test results showed a significant difference in antibody titer between the 100 mg/kg BW group and the positive control group ($p \leq 0.05$) from the normal control group while there was no significant difference ($p \geq 0.05$) from the 200 and 400 mg/kg BW groups. There was no significant difference between all the tested groups and the negative control group. However, only the positive control group exhibited a significant increase in antibody titter in comparison with the negative control group. This suggests that administration of QHPP extract at a dose of 100 mg/kg BW may increase antibody levels. Humoral immunity involves the interaction of B cells with antigens, leading to the proliferation and differentiation of B cells into plasma cells which secrete antibodies.[35] The assays show an increase in the response of the macrophages, a subset of T and B lymphocytes involved in antibody synthesis upon the administration of *S. costus* herbal product.

The ability of the QHPP extract to reduce edema in carrageenan-induced rat paws was measured to observe its anti-inflammatory effect. The assays showed that QHPP extract provided an anti-inflammatory effect at all the concentrations tested, and the dose of 75 mg/kg BW was the most effective in inhibiting the formation of edema since the effect was stable in every hour, and it had the highest percentage of edema inhibition (78.47% at the sixth hour) compared to the other doses. The percentage of rat paw edema and edema inhibition is described in Tables 6-7 and Figures 1-2. In several models of an anti-inflammatory assay, carrageenan-induction has been suggested for current protocols in pharmacology and in several articles to screen the active inflammatory compounds of a medicinal plant.[35-37] Carrageenan induced to the sub-plantar surface of rat paws causes biphasic edema.[38] During the first hour, the body releases histamine, serotonin, bradykinin, and a small number of prostaglandins produced by the cyclooxygenase (COX) enzyme. After one hour, there is a delayed phase with neutrophil infiltration and continued prostaglandin formation.[39] This recent study has shown that the herbal product of Qust al-Hindi has a potential COX inhibitory effect at the doses tested, which is as effective as the standard anti-inflammatory drug (diclofenac). The anti-inflammatory effect of a medicinal plant is closely linked to its chemical compounds. Some studies have shown that dehydrocostus lactone has a potential anti-inflammatory effect.[40-41] Meanwhile, the

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GC-MS instrument identified dehydrocostus lactone as the compound with the highest percentage area in the QHPP extract.

Table 6. The average % of rat paw Edema

Groups	Average % of rat paw edema± SD						
	Pre drug	1	2	3	4	5	6
NR	0±0	0±0	0±0	0±0	0±0	0±0	0±0
NT	0±0	72.22±13.61	106±49	131±60	161±71	197±78	200±52
R	0±0	44.44±17.21*	55.56±17.21*	83.33±18.26*	94.44±13.61*	72.22±13.61*	50±18.26*
D1	0±0	33.33±21.08*	38.89±13.61*	44.44±17.21*	61.11±25.09*	77.78±17.21*	77.78±27.22*
D2	0±0	31.94±21.35*	38.89±32.77*	52.78±36.77*	58.33±28.87*	52.78±30.12*	43.06±28.10*
D3	0±0	72.22±25.09	72.22±13.61	88.89±27.22*	100.00±29.81*	77.78±17.21*	83.33±18.26*

n=6, data are expressed as mean ± SEM, NR: Normal, NT: Negative, R: Reference, D1: Dose 1 (25 mg/kgBW), D2: Dose 2 (75 mg/kg BW), D3: Dose 3 (150 mg/kgBW)

Table 7. The Average Percentage of Edema Inhibition

Groups	% Inhibition of Edema(h)					
	1	2	3	4	5	6
NR	0±0	0±0	0±0	0±0	0±0	0±0
NT	0±0	0±0	0±0	0±0	0±0	0±0
R	38.46	47.59	36.39	41.34	63.34	75.00
D1	53.84	63.31	66.07	62.07	60.52	61.11
D2	55.77	63.31	59.71	63.77	73.21	78.47
D3	15.38	31.87	36.39	37.89	60.52	58.33

n=6, NR: Normal, NT: Negative, R: Reference, D1: Dose 1 (25 mg/kgBW), D2: Dose 2 (75 mg/kgBW), D3: Dose 3 (150 mg/kgBW)

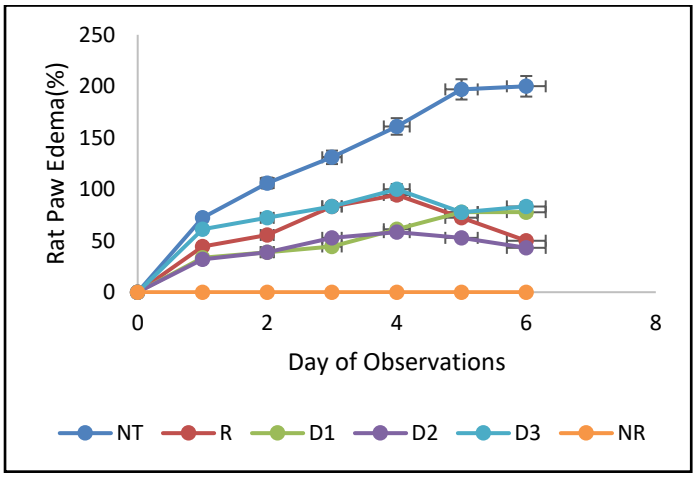

Figure 1: Percentage of Rat Paw Edema of Ethanol Fraction of QHPP Extract and Standard

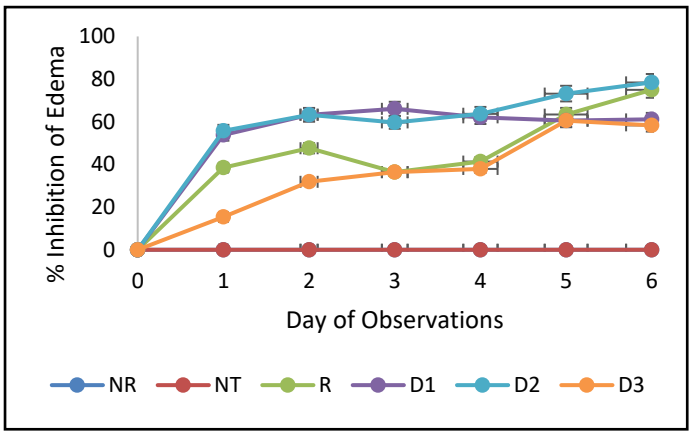

Figure 2: Percentage of Edema Inhibition of Ethanol Fraction of QHPP Extract and Standard

The QHPP extract was subjected to a preliminary cytotoxicity test using the Brine Shrimp Lethality Test (BSLT). As shown in Table 8, the BSLT results indicated that the extract at concentrations of 1000, 500, 250, 125 and 62.5, 31.25 ppm was lethal to *A. salina* larvae. In addition to the BSLT method, a cytotoxicity assay was further carried out with the colorimetric Micro tetrazolium (MTT) method.[26] The extract had the lowest absorbance against A549 cells at a concentration of approximately 500 ppm, with increasing absorbance at lower concentrations. This indicates that A549 cell viability declines with decreasing extract concentrations. The assay revealed that the crude extract had an IC_{50} of 279.39 ppm. Based on these results, the extract is considered toxic to A549 cells. The toxicity classification of the tested QHPP crude extract compared with the standards modified from those of NCI and Geran et al. ($IC_{50} \leq 20$ µg/mL = highly active, IC_{50} 21 – 200 µg/mL = moderately active, IC_{50} 201 – 500 µg/ml = weakly active, IC_{50} > 501 µg/ml = inactive) indicates that the crude extract is moderately active.[27]

In addition, regarding its antioxidant activity, the ethyl acetate fraction of QHPP offers a better free radical scavenging activity than any other fractions tested. All the fractions showed antioxidant activity. However, the ethyl acetate fraction showed the highest level of activity.

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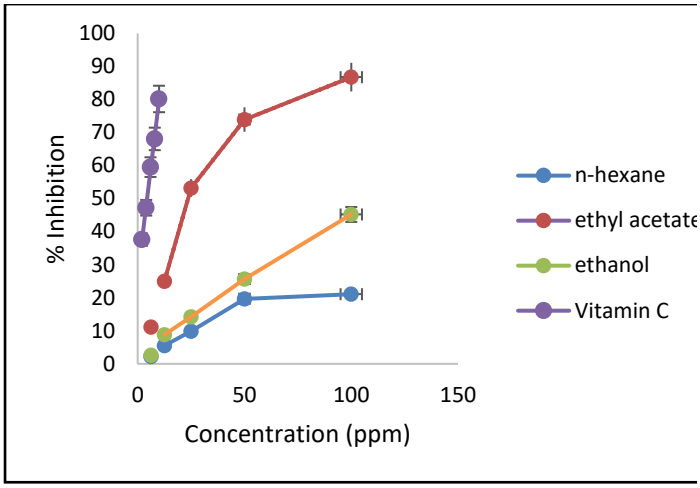

Figure 3: DPPH radical scavenging assay of Qusthul Hindi extract and vitamin C (Reference)

Table 8: Cytotoxicity Evaluation of QHPP extract with the BSLT and MTT Method

Concentration µg/ml	BSLT Method		MTT Method		
	% Death (Mean±SD)	LC_{50} (µg/ml, n=3)	Mean±SD	% Inhibition	IC_{50} (µg/ml, n=3)
500	100±0.0	57.75	0.242±0.071	67.95	279.39
250	100±0.0		0.258±0.012	65.78	
125	97±0.6		0.488±0.015	35.32	
62.5	60±0.0		0.660±0.002	12.47	
31.25	20±0.0		0.630±0.055	16.49	
Cell control	0		0.754±0.006	0.0	

Control: Penicilllin l00 U/mL, Streptomycin 100 ug/mL.

Conclusion

The study showed that QHPP has pharmacological activities similar to the native plant and can be utilized as a therapeutic supplement. In addition, it has been confirmed to be toxic at the acute toxicity level. Surprisingly, the herbal product was found to be cytotoxic against BSLT and A549 cell lines, and this provides an opportunity for further studies on its anticancer activity.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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