INVESTIGATION OF SOME BIOLOGICAL ACTIVITIES OF *DIOSCOREA WALLICHII* HOOK. F. (KADAT) TUBER

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Abstract

This study investigates the antimicrobial, antioxidant, cytotoxic, and anti-arthritic activities of *Dioscorea wallichii* Hook. L. (Kadat) tuber. The antimicrobial activity was screened using the agar well diffusion method on 95 % ethanol, watery, ethyl acetate, and pet-ether extracts. The results indicated that the watery extract exhibited the highest antimicrobial potency among the tested extracts. The *in vitro* antioxidant activity was assessed using the DPPH free radical scavenging assay, yielding IC_{50} values of 26.21 µg/mL for the watery extract and 13.62 µg/mL for the 95 % ethanol extract, indicating a stronger antioxidant effect from the ethanol extract. The anti-arthritic activity was evaluated by the egg albumin denaturation method, revealing that the 95% ethanol extract (IC_{50} = 6.77 µg/mL) exhibited more effective anti-arthritic activity than the watery extract (IC_{50} = 13.07 µg/mL). Cytotoxicity was assessed using a brine shrimp lethality bioassay, which demonstrated that both watery and 95 % ethanol extracts were free from toxicity at concentrations up to 1000 µg/mL, with LC_{50} values indicating no harmful effects.

Keywords: *Dioscorea wallichii* Hook. f. (Kadat) tuber, antimicrobial activity, antioxidant activity, anti-arthritic activity, cytotoxicity

Introduction

The genus *Dioscorea* L., within the family Dioscoreaceae, encompasses approximately 600 species distributed primarily across tropical and subtropical regions in Africa, Asia, and the Americas. Known for its ecological adaptability, Dioscorea includes herbaceous monocotyledonous plants, many of which are cultivated for their tubers, a major carbohydrate source in traditional diets. These tubers, rich in starch, play an essential role in providing daily caloric intake for communities in Africa and Asia and hold considerable economic significance (Ayensu & Coursey, 1972; Paul *et al*., 2017). Dioscorea plants, including commonly known yams, also contribute to the pharmaceutical and cosmetics industries, notably as a source of diosgenin, a steroidal sapogenin used in the synthesis of steroid drugs. Distinctive for their twining, climbing stems, which may coil in either direction, Dioscorea species exhibit alternate or occasionally opposite leaves, often cordate with prominent venation. Species such as *D. wallichi*i, distributed throughout regions like Bangladesh, China, and Southeast Asia, are prized not only as food but also for their therapeutic properties. Indigenous communities have long valued Dioscorea for traditional medicine, with uses ranging from pain relief and treatment of dysentery to skin ailments and rheumatism, though comprehensive documentation of these practices remains limited (Kumar *et al*., 2017). Bioactive compounds found in Dioscorea spp., such as alkaloids, flavonoids, saponins, and phenols, contribute to a wide range of health benefits, including antioxidant, anti-inflammatory, antimicrobial, and antiviral effects (Salehi *et al*., 2019). Specifically, steroidal saponins in Dioscorea have shown potential in cancer prevention and immune support (Patel *et al*., 2012).

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The therapeutic potential of *D. wallichii*, particularly due to compounds like diosgenin, has drawn interest in reproductive health applications and hormone therapy (Che *et al*., 2014). Dioscorea is important in nutrition and medicine, making it a valuable subject of ethnobotanical and pharmacological research as both a cultural staple and a promising health resource.

Materials and Methods

Plant Materials

Kadat tubers were collected from July to August 2024 in Taikkyi Township, Yangon Region, Myanmar. (Figure 1) The collected sample was identified as *Dioscorea wallichii* Hook. L. (Kadat) tuber at the Botany Department, University of Yangon. The sample was cleaned thoroughly with water. Then the fresh tuber was cut into small pieces and air-dried at room temperature. The dried samples were ground into powder by a grinding machine, sieved and stored in an airtight container for further use.

Figure 1. Photographs of plant and tuber of *D. wallichii*

Preparation of Crude Extracts for Biological Activities

Approximately 20 g of dried powdered sample was extracted three times with 95% ethanol for 6 h, then filtered, and the volume was reduced using a rotary evaporator to obtain the ethanol extract. For the water extract, 20 g of dried powdered sample was boiled in 100 mL of distilled water, then filtered, and the solvent was concentrated by evaporation in a water bath. This produced the water extract. Other crude extracts were prepared following the same procedure as for the 95% ethanol extract. All crude extracts were dried and stored in a refrigerator for future use.

Determination of Antimicrobial Activity by Agar Well Diffusion Method

For antimicrobial activity screening by the agar well diffusion method, 0.5 g of peptone and 0.25 g of sodium chloride were dissolved in distilled water, and the solution was made up to 100 mL with additional distilled water. The pH of this solution was adjusted to 7.2 using 0.1 M sodium hydroxide, and 1.5 g of agar was added. The nutrient agar was then boiled, and 20–25 mL of the medium was poured into a test tube, plugged with cotton wool, and autoclaved at 121 °C for 15 min. The tubes were subsequently cooled to 60 °C, and the medium was poured into sterilized Petri dishes, followed by the addition of 0.1 mL of spore suspension. The agar was

allowed to set for 30 min, after which a 10 mm agar well was made using a sterilized cork borer. Next, approximately 0.1 mL of the sample was introduced into the agar well, and the plates were incubated at 37 °C for 24 h. The antimicrobial activity of ethanol, water, ethyl acetate, and petroleum ether extracts of Kadat tuber was screened using the agar well diffusion method. The appearance of an inhibition zone around the agar well indicated the presence of antimicrobial activity.

Determination of Antioxidant Activity by DPPH Free Radical Scavenging Assay

The DPPH free radical scavenging activity of 95% ethanol and water extracts of Kadat tuber was determined using a UV-visible spectrophotometric method. The control solution was prepared by mixing 1.5 mL of 20 µM DPPH solution with 1.5 mL of 95% ethanol in brown bottles, using a shaker. The blank solution was prepared by mixing 1.5 mL of the test sample solution with 1.5 mL of 95% ethanol. The sample solutions were prepared by thoroughly mixing 1.5 mL of 20 µM DPPH solution with 1.5 mL of various concentrations of test sample solution (3.91, 7.81, 15.63, 31.25, 62.50, and 125.00 µg/mL). These solutions were incubated at room temperature and shaken for 30 min. The absorbance of each solution was then measured at 517 nm using a UV-visible spectrophotometer (Irulandi *et al*., 2016). The absorbance measurements were performed in triplicate for each concentration.

Determination of Cytotoxicity by Brine Shrimp Lethality Bioassay

The brine shrimp (*Artemia salina*) were used in this study for a cytotoxicity bioassay. Artificial seawater [3.8% (w/v) NaCl] was prepared by dissolving 38 g of sodium chloride in 1 L of distilled water. Brine shrimp cysts (0.25 g) were put into 500 mL of artificial seawater in a separating funnel. This funnel was placed near a lamp. Light is essential for the cysts to hatch. Brine shrimp cysts are required to hatch with constant supplied oxygen and 24 h incubation at room temperature. 9 mL of artificial seawater and 1 mL of different concentrations of samples (1000, 100, 10, 1 µg/mL) and standard solutions were added to each chamber. Alive brine shrimp (10 nauplii) were then taken with a Pasteur pipette and placed into each chamber. They were incubated at room temperature for about 24 h. After 24 h, the number of dead and surviving brine shrimp was counted, and cytotoxicity was estimated using the 50 % lethal concentration (LC_{50}) (Singh *et al*., 2015). The control solution was prepared following the same procedure, using distilled water instead of the sample solution.

Determination of Anti-arthritic Activity by Egg Albumin Method

The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from a fresh hen's egg), 2.8 mL of phosphate-buffered saline (PBS, pH 6.4), and 2 mL of varying concentrations of crude Kadat tuber extracts (95 % ethanol and watery extracts) to achieve final concentrations of 100, 200, 400, 800, and 1000 µg/mL. A similar volume of double-distilled water was used as the control. The mixtures were then incubated at 37°C for 15 min, followed by heating at 70 °C for 5 min. After cooling, the absorbance was measured at 660 nm. Diclofenac sodium was used as the standard drug (Sunmathi *et al*., 2016).

Results and Discussion

Antimicrobial Activity of Extracts of Kadat Tuber by Agar Well Diffusion Method

The antimicrobial activity of four crude extracts: petroleum ether, ethyl acetate, 95% ethanol, and water, derived from *Dioscorea wallichii* Hook. f. (Kadat) tuber was assessed by using the agar well diffusion method against a panel of eight microorganisms: *Agrobacterium tumefaciens*, *Bacillus pumilus*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. This method, which measures the diameter of inhibition zones around each well, is an established technique for evaluating antimicrobial potency; a larger zone of inhibition indicates stronger antimicrobial activity. The study results, summarized in Table 1 and illustrated in Figure 2, revealed that among the extracts tested, the water extract demonstrated the highest antimicrobial efficacy across the tested microorganisms. The superior activity of the water extract could be attributed to its ability to concentrate polar bioactive compounds, which are more soluble in aqueous media and may include antimicrobial phytochemicals such as saponins, tannins, or glycosides commonly found in *Dioscorea* species. The extracts of ethanol, ethyl acetate, and petroleum ether showed moderate to low antimicrobial activity, varying due to their solubility of active constituents in solvents of varying polarities.

Table 1. Inhibition Zone Diameter of Crude Extracts from *D. wallichii* Tuber by Agar Well Diffusion Method

Microorganisms	Diameter of inhibition zone (mm)				
	PE	**EtOAc**	**EtOH**	**H_2O**	***STD**
A. tumefaciens	15	16	19	21	27
B. pumilus	16	16	19	21	27
B. subtilis	15	16	19	22	27
C. albicans	15	16	19	21	27
E. coli	16	16	19	22	27
M. luteus	15	16	19	22	28
P. fluorescens	16	16	20	22	28
S. aureus	15	16	19	21	28

For Bacterial *STD = Chloramphenicol
For Fungus *STD = Nystatin

Agar well diameter – 10 mm
10 mm – 14 mm = weak activity
15 mm – 19 mm = moderate activity
20 mm and above = potent activity

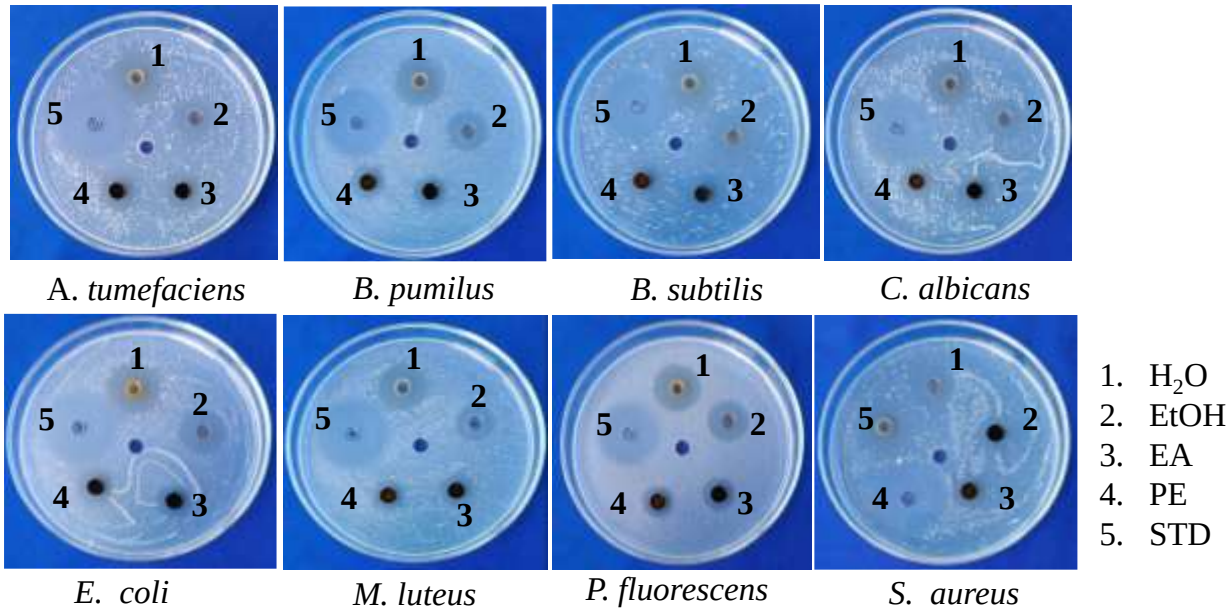

Figure 2. Antimicrobial activity of various crude extracts from the tuber of *D. wallichii* (Kadat) on eight microorganisms

Antioxidant Activity of Extracts of Kadat Tuber by DPPH Free Radical Scavenging Assay

The antioxidant activity of ethanol and watery extracts from the Kadat tuber was evaluated using the DPPH free radical scavenging assay, with ascorbic acid serving as the standard reference. The extracts were tested with six different concentrations: 3.91, 7.81, 15.63, 31.25, 62.5, and 125 µg/mL. The absorbance was measured at 517 nm using a UV-visible spectrophotometer. The results indicated that the IC_{50} values for the ethanol extract, aqueous extract, and ascorbic acid were 13.62 µg/mL, 26.21 µg/mL, and 4.68 µg/mL, respectively. This suggests that the ethanol extract exhibited a more potent antioxidant activity compared to the watery extract. However, both crude extracts demonstrated lower antioxidant activity than the standard ascorbic acid. Overall, the findings indicate that the Kadat tuber possesses significant free radical scavenging activity, suggesting its potential as an alternative source for cancer treatment. Detailed results can be found in Table 2 and illustrated in Figures 3 and 4.

Table 2. Average % Inhibition and IC_{50} Values of Crude Extracts of Kadat Tuber

Extract	% RSA ± SD at different concentrations (µg/mL)						IC_{50} (µg/mL)
	3.91	7.81	15.62	31.25	62.5	125	
ethanol	35.5 ± 0.44	42.12 ± 0.48	52.72 ± 0.42	65.69 ± 0.00	71.97 ± 0.75	78.24 ± 0.55	13.62
watery	23.78 ± 0.44	29.92 ± 0.96	44.00 ± 0.74	52.86 ± 0.53	59.07 ± 1.03	66.53 ± 1.16	26.21
std. Ascorbic acid	48.99 ± 0.53	54.09 ± 1.36	56.64 ± 0.44	63.83 ± 1.06	66.61 ± 0.35	87.13 ± 0.76	4.68

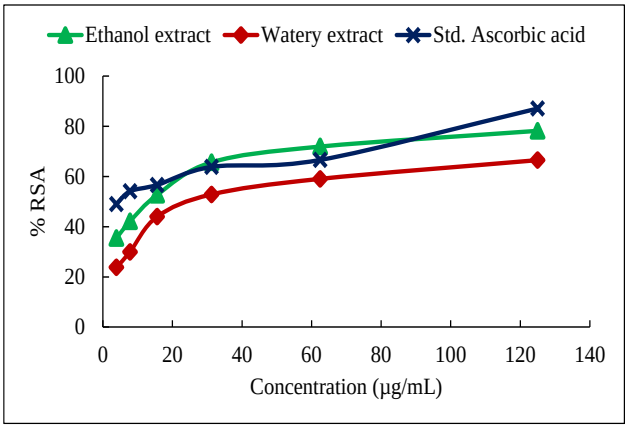

Figure 3. A plot of % RSA of different concentrations of crude extracts of Kadat tuber

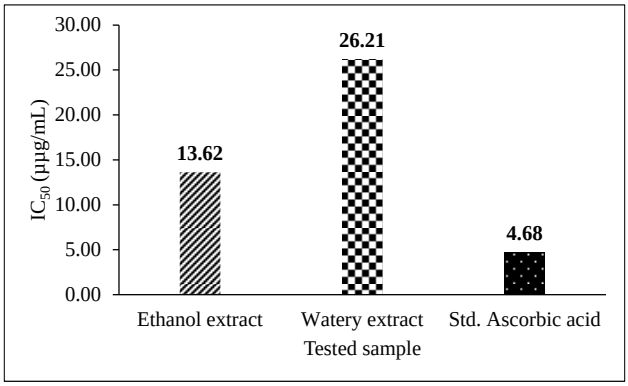

Figure 4. A bar graph of IC_{50} values of crude extract of Kadat tuber and standard ascorbic acid

Anti-arthritic Activity of Crude Extracts of Kadat Tuber

Arthritis, a chronic inflammatory disorder, often results from the denaturation of proteins, which triggers immune responses and leads to symptoms such as joint pain, swelling, and stiffness. The anti-arthritic potential of natural products, particularly plant extracts, is commonly evaluated by measuring their ability to inhibit protein denaturation, which serves as an indicator of their anti-inflammatory and immunomodulatory effects. In this study, the anti-arthritic activity of ethanol and water extracts of Kadat tuber was assessed *in vitro* using the protein denaturation inhibition method with egg albumin as a model protein.

The inhibitory concentration (IC_{50}) values of the ethanol and water extracts against protein denaturation were 6.77 µg/mL and 13.07 µg/mL, respectively (Table 3). These values represent the concentrations required to inhibit 50% of protein denaturation and serve as a quantitative measure of the effectiveness of extract. A lower IC_{50} value indicates a higher potency in inhibiting protein denaturation. Consequently, the ethanol extract of Kadat tuber, with its lower IC_{50}, demonstrates ~~superior~~ anti-arthritic activity compared to the standard and the water extract (Figures 5 and 6). This enhanced efficacy of the ethanol extract could be attributed to its potential to dissolve and concentrate more bioactive compounds that may be responsible for reducing protein denaturation.

Table 3. Average % Protein Denaturation and IC_{50} Values of Crude Extracts of Kadat Tuber

Extract	% Protein denaturation at different concentrations (µg/mL)						IC_{50} (µg/mL)
	3.91	7.81	15.62	31.25	62.5	125	
Ethanol	34.34 ± 9.50	55.68 ± 0.59	76.54 ± 0.39	80.66 ± 0.10	86.38 ± 0.10	89.64 ± 0.18	6.77
Watery	30.52 ± 2.86	41.97 ± 0.93	53.9 ± 2.37	65.73 ± 1.64	74.92 ± 0.12	84.84 ± 0.14	13.07
std. diclofenac	8.41 ± 1.93	45.69 ± 0.85	53.23 ± 0.00	60.26 ± 0.66	70.28 ± 1.14	79.39 ± 0.75	12.28

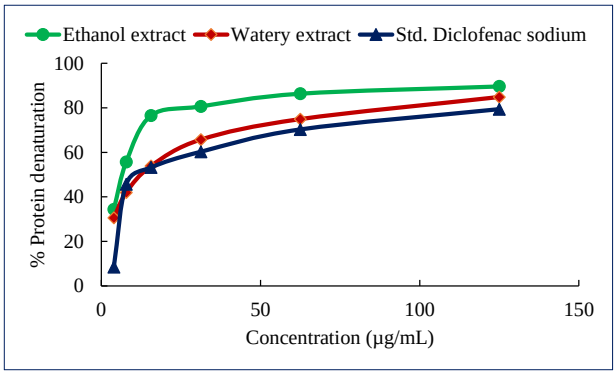

Figure 5. A plot of % protein denaturation versus concentrations of crude extracts of Kadat tuber and standard diclofenac sodium

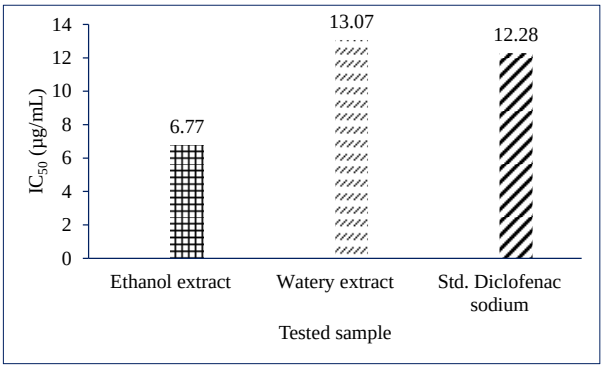

Figure 6. A bar graph of IC_{50} values of crude extracts of Kadat tuber and standard diclofenac sodium

Cytotoxicity of Crude Extracts of Kadat Tuber

The cytotoxicity of the water and ethanol extracts of *D. wallichii* Hook. f. (Kadat) tuber was evaluated using the brine shrimp lethality bioassay, a well-established preliminary screening method to assess potential toxicity. In this assay, brine shrimp (*Artemia salina*) larvae are exposed to varying concentrations of crude extracts to determine their lethal concentration (LC_{50}). LC_{50} values below 1000 µg/mL are considered toxic (active), while values above 1000 µg/mL indicate non-toxic (inactive) extracts. According to the results summarized in Table 4, the LC_{50} values of both the water and ethanol extracts were greater than 1000 µg/mL, suggesting that neither extract exhibited cytotoxic effects under the conditions tested. These findings indicate that both extracts of Kadat tuber are likely safe and non-toxic at the concentrations used in the assay. The absence of cytotoxicity in the brine shrimp lethality bioassay suggests that the water and ethanol extracts of Kadat tuber may be safe for further development and use, particularly in applications involving food or medicinal formulations. However, while the brine shrimp assay provides an initial indication of cytotoxicity, further *in vitro* and *in vivo* cytotoxicity studies on human cell lines would be necessary to comprehensively evaluate the safety profile of these extracts for potential therapeutic or dietary use.

Table 4. Cytotoxicity of Different Concentrations of Crude Extracts of Kadat Tuber Against *Artemia salina* (Brine Shrimp)

Extract	Dead % by using different concentrations (µg/mL) of samples (Mean ± SEM)				LC_{50} (µg/mL)
	1	10	100	1000	
Ethanol	30 ± 0.00	40 ± 0.00	40 ± 0.00	47 ± 0.00	> 1000
Watery	33 ± 0.00	37 ± 1.53	40 ± 0.71	40 ± 0.00	> 1000
$K_2Cr_2O_7$	7 ± 1.00	20 ± 3.01	100 ± 0.00	100 ± 0.00	43.75
Caffeine	0 ± 0.00	23 ± 1.20	30 ± 0.58	40 ± 1.16	> 1000

Conclusion

The present research investigates various biological activities of the Kadat tuber, focusing on its antimicrobial, antioxidant, anti-arthritic, and cytotoxicity effects. The watery extract exhibited potent antimicrobial activity, with inhibition zone diameters exceeding 20 mm while the ethanol, ethyl acetate, and pet-ether extracts showed moderate activity, with inhibition zones ranging from 15 to 19 mm. The antioxidant activity in terms of IC_{50} values is 13.62 µg/mL for ethanol, 26.21 µg/mL for watery extract and 4.68 µg/mL for ascorbic acid. The ethanol extract exhibited higher antioxidant activity than the watery extract indicating its free radical scavenging properties. In the anti-arthritic activity, the ethanol extract (IC_{50} = 6.77 µg/mL) was more effective than the aqueous extract (IC_{50} = 13.07 µg/mL). Regarding cytotoxicity, both ethanol and watery extracts of the Kadat tuber showed no cytotoxic effects on brine shrimp at concentrations up to 1000 µg/mL. The findings of this research highlight the significant biological activities of the Kadat tuber, particularly its potent antimicrobial and anti-arthritic properties. The ethanol extract emerged as the most effective in both antioxidant and anti-arthritic activities, while the aqueous extract showed promising antimicrobial effects. In experiments, neither extract was harmful to cells at the concentrations tested, which suggests they might be safe for further applications. Overall, the Kadat tuber represents a valuable natural resource with potential therapeutic investigation to explore its full range of bioactive compounds.

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