INVESTIGATION OF ANTIOXIDANT, ANTIMICROBIAL, AND CYTOTOXIC ACTIVITIES OF HEARTWOOD OF *MANSONIA GAGEI* J. R. DRUMM. (KALA-MET)

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Abstract

The heartwood of *Mansonia gagei*. locally known as Kala-met, was studied for its antioxidant, antimicrobial, and cytotoxic properties, which are used in treating heart disease and diabetes. The DPPH free radical scavenging assay revealed that the ethanol extract (IC_{50} 63.88 μg/mL) exhibited higher antioxidant potency than the watery extract (IC_{50} 157.75 μg/mL). However, they were found to have weaker antioxidant potency than standard ascorbic acid (IC_{50} 3.33 μg/mL). The antimicrobial activity of the four crude extracts, such as watery, ethanol, ethyl acetate, and pet-ether extracts, was screened by the Agar well diffusion method using eight pathogenic strains: *Agrobacterium tumefaciens, Bacillus pumilus, Bacillus subtilis, Candida albicans, Escherichia coli, Micrococcus luteus, Pseudomonas fluorescens, and Staphylococcus aureus*. All tested crude extracts exhibited medium to high antimicrobial activity, with inhibition zone diameters ranging between 14 and 26 mm on all strains. In addition, the cytotoxicity of ethanol and watery extracts was conducted using a brine shrimp lethality bioassay, indicating that both watery and ethanol extracts showed cytotoxic activity on brine shrimp with LC_{50} values < 1000 µg/mL, a medium-toxic effect.

Keywords: *Mansonia gagei* J. R. Drumm. (Kala-met), antioxidant, antimicrobial and cytotoxic activities

Introduction

Our country, Myanmar, possesses a rich resource of medicinal plants, which are widely practiced by the majority of the population. However, there are limited pharmacological studies on traditional medicines. One of the medicinal plants, *Mansonia gagei* J. R. Drumm., commonly known as Kala-met, is widely grown in Tanintharyi, Myanmar (Figure 1). It has a bitter and cold taste and a pleasant fragrance. In Myanmar's traditional medicine (TM 15), it is extensively used to treat bile issues, anxiety, and heart disease. The cold extract of *M. gagei* is commonly used externally to regulate body temperature and orally to reduce the menstrual cycle for women going through the menopause. It belongs to the family Sterculiaceae and has been used as a cardiac stimulant, liver antiemetic, antidepressant, and refreshment agent. Certain reports on the chemical constituents of the Mansonia genus were addressed. The heartwood of *M. gagei* constituted primarily 1,2-naphthoquinones of the mansonone type: mansonones A–H (Bettòlo et al., 1965), mansonones I and L (Galeffi et al., 1969), tetrahydropalmatine, crebanine, *O*-methylbulbocapnine, and *N*-methyltetrahydropalmatine. The aim of the present research is to screen the antioxidant, antimicrobial, and cytotoxic activities of the heartwood of *M. gagei* (Kala-met).

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Figure 1. The photograph of heartwood of *M. gagei* (Kala-met)

Materials and Methods

Collection and Identification of Plant Sample

The heartwood of *M. gagei* (Kala-met) was collected from Dawei Township, Tanintharyi Region, Myanmar, in February 2024. Its botanical name was identified at the Department of Botany, University of Yangon. The sample was made into small pieces and air-dried at room temperature, and then ground into a fine powder and stored in an airtight container.

Chemicals Requirements

2,2-diphenyl-1-piccry-hydrazyl (DPPH), ascorbic acid, trypticase soy broth from Difco U.S.A, trypticase soy agar Becton U.S.A, Mueller-Hinton agar (Hi-media), triple sugar, iron sugar (TSI) agar Becton U.S.A, pet-ether, ethyl acetate, ethanol, sodium chloride, potassium dichromate, caffeine, dimethyl sulphoxide, artificial seawater (37%)

Instruments Requirements

Quartz cuvette (4 mL), UV-visible spectrophotometer (UV-7504), electronic balance, a stirrer, an autoclave (Tomy Seiko Co., Ltd, Tokyo, Japan), a constant temperature waterbath (Yamato Scientific Co., Ltd, Japan), sterile Petri, spirit burner, polyethylene plastic bag, a refrigerator, an incubator, multipipette, 96-well plate, aluminium foil, centrifuge tube, haemacytometer, microscope, vibrator, syringe (3 mL, 5 mL), beakers, chambers, Pasteur pipette, lamp, and water bottles

Preparation of Crude Extracts from the Heartwood of *M. gagei*

Each 50 g of air-dried powder sample was percolated with 200 mL each of pet-ether, ethyl acetate, ethanol, and water for 48 h at room temperature and then filtered. After filtration, the respective filtrate was evaporated under reduced pressure by a rotary evaporator to yield different crude extracts.

Determination of Antioxidant Activity of Crude Extracts by DPPH Assay

The antioxidant activity of ethanol and watery extracts of *M. gagei* was determined by the DPPH (2,2-diphenyl-1-picryl-hydrazyl) free radical scavenging assay, according to the procedure described by (Maisarah et al., 2013). DPPH powder (4.2 mg) was thoroughly dissolved in 100 mL of EtOH to get a120 µM DPPH solution. This solution was freshly prepared in the brown- coloured reagent bottle and stored in the fridge for no longer than 24 hours before use. The stock solution (40 µg/mL) of standard ascorbic acid was prepared by dissolving 4 mg of the compound in 10 mL of ethanol. Each extract, 40 mg, and 50 mL of ethanol were thoroughly mixed. The sample solutions (800, 400, 200, 100, 50, 25, 12.5, and 6.25 µg/mL concentrations) were prepared by diluting with an appropriate amount of ethanol. The control solution was created by mixing 1.5 mL of DPPH solution and 1.5 mL of ethanol. The sample solution was prepared by mixing 1.5 mL of DPPH solution and 1.5 mL of sample solution. The blank solution was created by mixing 1.5 mL of sample solution and 1.5 mL of ethanol. These solutions were prepared in the brown bottles and then incubated at room temperature and shaken on a shaker for 30 minutes. The absorbance of the prepared solutions was measured at 517 nm using a UV-visible spectrophotometer. The percent radical scavenging activity was calculated by the following equation.

$$\% \text{ RSA} = \{A_{DPPH}\text{-}(A_{\text{ sample}} - A_{\text{ blank}})/A_{DPPH}\} \times 100$$

Where,

% RSA = percent radical scavenging activity

A_{DPPH} = absorbance of DPPH in ethanol solution

$A_{\text{ sample}}$ = absorbance of sample + DPPH solution

$A_{\text{ blank}}$ = absorbance of sample + EtOH solution

The antioxidant power (IC_{50}) is expressed as the test substance concentration (µg/mL) that results in a 50% reduction of the initial absorbance of the DPPH solution and allows for determination of the concentration. IC_{50} (50% inhibitory concentration) values were calculated by using a linear regressive Excel program.

$$\text{Standard Deviation (SD)} = \sqrt{\frac{(\bar{x} - x_1) + (\bar{x} - x_2)^2 + \ldots(\bar{x} - x_n)^n}{(n-1)}}$$

Evaluation of Antimicrobial Activity of Crude Extracts by Agar Well Diffusion Method

The agar well diffusion method was used to evaluate the antimicrobial activity of the four crude extracts, including PE, EtOAc, EtOH, and H_2O extracts, of the heartwood of *M. gagei* using 8 pathogenic strains. Different crude extracts (1 mg each) were dissolved in 1 mL of their respective solvents. After inoculation, plates were dried for 15 min. A hole with a diameter of 8 mm was punched aseptically with a sterile cork or a tip, and 0.1 mL of extract solution at the desired concentration was introduced into the well. Then, agar plates were incubated under suitable conditions depending upon the microorganisms. The antimicrobial agent inhibited microbial growth in agar medium after 24 h of incubation, forming zones of inhibition as it diffused through the media. The experiments were conducted at Pathein University. The observation of the inhibition zone diameters and the measurements were recorded.

Determination of Cytotoxicity of Crude Extracts by Brine Shrimp Lethality Bioassay

The cytotoxicity of ethanol and watery extracts of *M. gagei* was investigated by a brine shrimp lethality bioassay, according to the procedure described by Olowa *et al*. (2013). The brine shrimp (*Artemia salina*) was used in this bioassay (Ali *et al.,* 2013). The sample solution was prepared by dissolving 5 mg of the respective crude extract in 5 mL of distilled water. The stock solution was tenfold diluted serially with distilled water to get the sample solutions with concentrations of 800, 400, 200, 100, and 50 µg/mL. Artificial seawater (9 mL) was mixed with the sample solution (1 mL) and placed in the chamber of the ice cup. Alive brine shrimp (10 nauplii) were taken with a Pasteur pipette and then placed into each chamber, which was kept at room temperature for about 24 h. After incubation, the number of surviving brine shrimp was counted, and a 50% lethal dose (LC_{50}) was calculated (Deciga-Campos, *et al*.,2007). The cytotoxic effect of control solutions ($K_2Cr_2O_7$ and caffeine) was also determined according to the above procedure (Clarkson *et al*., 2004).

Results and Discussion

Antioxidant Activity of Crude Extracts

Ethanol and watery extracts of *M. gagei* heartwood were tested for antioxidant activity using a DPPH free radical scavenging assay. A UV spectrophotometer was used in this investigation to evaluate the absorbance at λ_{max} 517 nm of various concentrations (800, 400, 200, 100, 50, 25, 12.5, and 6.25 µg/mL for the tested sample and 40, 20, 10, 5, 2.5, and 1.25 µg/mL for the standard ascorbic acid). According to the experimental findings antioxidant activity ethanol extract (IC_{50} 63.88 µg/mL) was more potent than that of watery extract (IC_{50} 157.75 µg/mL). However, when compared to the strength of standard ascorbic acid (IC_{50} 3.33 µg/mL), the antioxidant activity of both extracts was found to be relatively small. These results are presented in Tables 1 and 2, and Figures 2 and 3.

Table 1. Radical Scavenging Activity (% RSA) and IC_{50} Values of Ethanol and Watery Extracts of *M. gagei* Heartwood

Extract	% RSA ± SD at Different Concentrations (µg/mL)								IC_{50} (µg/mL)
	6.25	12.5	25	50	100	200	400	800	
Ethanol	9.1 ± 0.002	14.3 ± 0.001	34.7 ± 0.01	46.5 ± 0.003	59.1 ± 0.002	71.1 ± 0.001	73.8 ± 0.004	78.7 ± 0.003	63.88
Water	5. 3 ± 0.004	9.6 ± 0.005	13.4 ± 0.002	34.0 ± 0.008	45.9 ± 0.003	53.0 ± 0.004	68.9 ± 0.002	71.4 ± 0.003	157.75

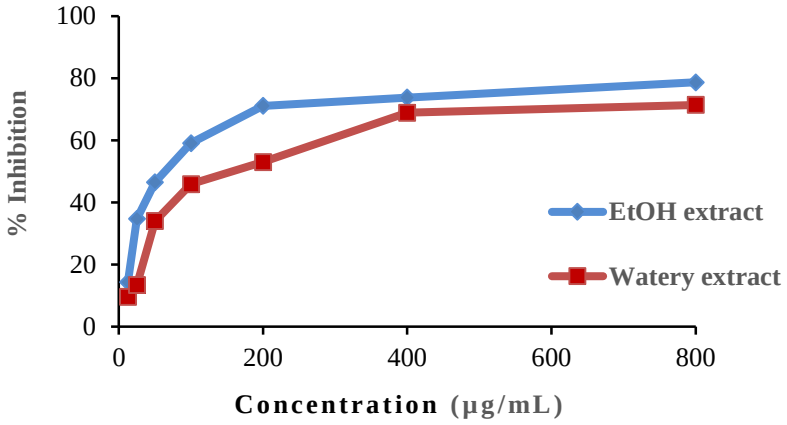

Figure 2. A plot of % RSA *vs* concentrations of ethanol and watery extracts of heartwood of *M. gagei*

Table 2. Radical Scavenging Activity (% RSA) of Standard Ascorbic Acid

Sample	% RSA ± SD at Different Concentrations (*µ*g/mL)						IC_{50} (*µ*g/mL)
	1.25	2.5	5	10	20	40	
Ascorbic acid	18.91 ± 0.05	38.70 ± 0.04	72.32 ± 0.018	98.76 ± 0.006	97.60 ± 0.00	96.31 ± 0.00	3.33

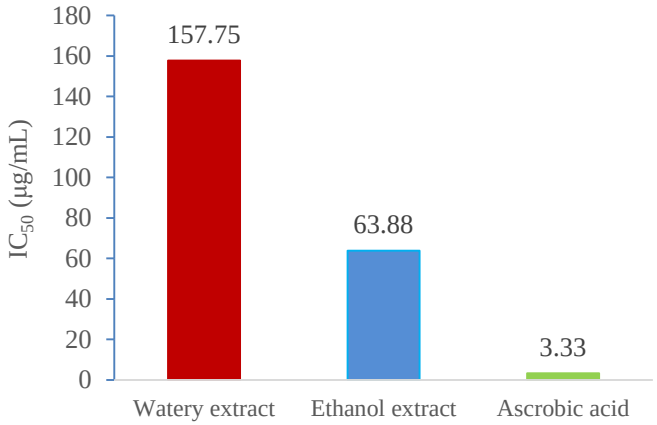

Figure 3. Histogram of IC_{50} values of watery and ethanol extracts of *M. gagei* and standard ascorbic acid

Antimicrobial Activity of Crude Extracts

The antimicrobial activity was determined by measuring the diameter of the zone of inhibition and recording it according to the method described by Selvamohan *et al*., 2012. In this investigation, four extracts (PE, EtOH, EtOAc, and H_2O) of *M. gagei* were tested using bacteria (*B. subtilis, E. coli, P. fluorescens, A. tumefaciens, B. pumilus, M. luteus,* and *S. aureus*) and *C. albicans* fungus by the agar well diffusion method. The measurable zone diameter, including the well diameter, shows the degree of completeness of the antimicrobial activity. In this experiment, the well diameter was set at 8 mm. The three crude extracts, EtOAc, EtOH, and H_2O, possessed

high activity on all tested organisms, with the inhibition zone diameter ranged between 20 and 26 mm by comparing with the standard drugs, chloramphenicol and nystatin. The PE extract show less activity (< 15 mm) than the other extracts The observed antimicrobial activity of the heartwood of *M. gagei* expressed as the zone of inhibition is shown in Table 3, and the photograph of the agar plate showing the inhibition zones is illustrated in Figure 4. Generally, the more susceptible the organisms, the bigger the zone of inhibition.

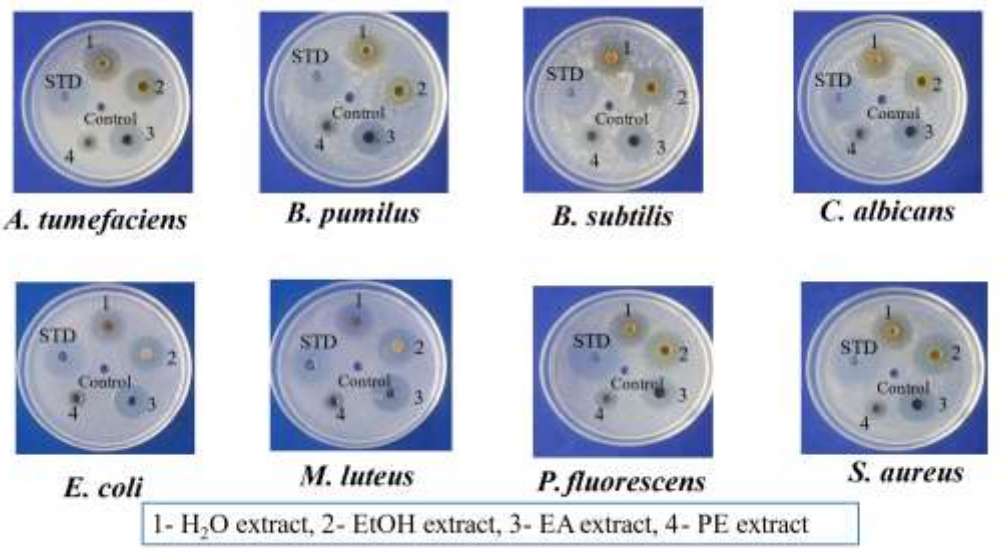

Figure 4. Antimicrobial activity of different crude extracts of *M. gagei* on eight microorganisms

Table 3. Inhibition Zone Diameters (mm) of Crude Extracts of *M. gagei* against Tested Microorganisms

Microorganisms	Inhibition Zone Diameters of Extracts (mm)				
	H_2O	EtOH	EA	PE	STD*
A. tumefaciens	25	25	25	14	27
B. pumilus	23	26	20	14	26
B. subtilis	22	23	23	14	27
C. albicans	24	23	23	14	27
E. coli	22	20	23	14	27
M. luteus	24	22	23	14	28
P. fluorescens	25	26	22	15	28
S. aureus	24	24	23	15	28

Agar well – 8 mm
10 mm ~ 14 mm (low activity)
15 mm ~ 19 mm (moderate activity)
20 mm above 9 (high activity)

*STD for bacteria = Chloramphenicol
for fungus = Nystatin

Cytotoxicity of Ethanol and Watery Extracts from the Heartwood of *M. gagei*

The cytotoxic activity of ethanol and watery extracts of heartwood of *M. gagei* was evaluated by a brine shrimp lethality bioassay. The nauplii were exposed to different concentrations of plant extracts for 24 hours. The number of motile nauplii was counted to determine the effectiveness of the extract. The cytotoxic effect was expressed as LC_{50} values (50 % lethal concentration). The potassium dichromate ($K_2Cr_2O_7$) and caffeine were chosen as a positive control and a negative control, respectively, because $K_2Cr_2O_7$ is a toxic agent in this assay and caffeine is a natural product. From the results, the LC_{50} values of ethanol and watery extracts were found to be 300 µg/mL and 608.89 µg/mL, and the standard potassium dichromate and caffeine were 89.98 µg/mL and > 1000 µg/mL, respectively. In the presence study, the two extracts exhibit moderate cytotoxic effects comparable to those of the standard potassium dichromate. Therefore, ethanol and watery extracts of *M. gagei*, were used as antioxidants for cancer chemoprevention. The experimental data are shown in Table 4 and Figure 5.

Table 4. Cytotoxicity of Ethanol and Watery Extracts of the Heartwood of *M. gagei*

Sample	% of Dead Brine Shrimp in Different Concentrations (µg/mL)					LC_{50} (µg/mL)
	50	100	200	400	800	
EtOH	26.67 ± 0.47	33.33 ± 0.57	46.66 ± 0.57	53.33 ± 0.40	63.33 ± 0.57	300
Watery	3.33 ± 0.47	16.67 ± 0.57	36.67 ± 0.82	43.33 ± 0.57	56.67 ± 0.57	608.89
*$K_2Cr_2O_7$	36.67 ± 0.53	53.34 ± 0.58	72.0 ± 1.0	85.47 ± 1.15	100 ± 0.00	89.98
**Caffeine	0.00± 0.00	0.00± 0.00	6.67 ± 0.57	16.67 ± 0.82	23.34 ± 0.54	>1000

*$K_2Cr_2O_7$ = Positive control
**Caffeine = Negative control

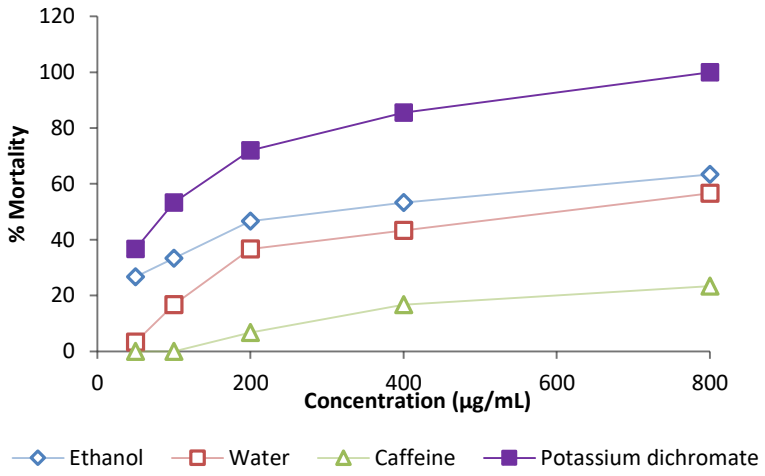

Figure 5. A plot of % mortality vs concentration of standards caffeine and potassium dichromate solutions, ethanol and watery extracts of the heartwood of *M. gagei*

Conclusion

In this research work, the screening of antioxidant, antimicrobial, and cytotoxic activities based on ethanol, ethyl acetate, petroleum ether, and watery extracts of the heartwood of *M. gagei* were carried out. The antioxidant activity of the ethanol and watery extracts was conducted by the DPPH free radical scavenging assay, and it was observed that the ethanol extract (IC_{50} 63.88 μg/mL) had more antioxidant potency than the watery extract (IC_{50} 157.75 μg/mL). All tested crude extracts showed antimicrobial activity on tested microorganisms with inhibition zone diameter ranges of 22–25 mm for watery extract, 20–26 mm for ethanol extract, 20–25 mm for ethyl acetate extract, and 14–15 mm for pet-ether extract. Especially, the ethanol extract had the highest activity on *B. pumilus* at 26 mm. The watery and ethanol extracts showed cytotoxic activity with LC_{50} values <1000 μg/mL by a brine shrimp lethality bioassay. From these results, it could be deduced that the heartwood of *M. gagei* possessed phytochemical constituents with potential pharmacological activities viz as antioxidant, antimicrobial, and cytotoxic activities. This finding provided potential for the heartwood of *M. gagei* as a therapeutic drug for traditional medicinal treatment.

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References

Ali, N., U. Alien, S. W. A. Shaha, M. Junaid, G. Ahmed, W. Ali, and M. Ghias. (2013). "Acute Toxicity, Brine Shrimp Cytotoxicity, Anthelmintic and Relaxant Potentials of Fruits of ***Rubus fruiticosis Agg***". *BMC Complement Altern. Med*., vol.13, pp.136-143.

Bettòlo, G. B. M., C. G. Casinovi, and C. Galeffi. (1965). "A New Class of Quinones: Sesquiterpenoid Quinones of *Mansonia altissima* Chev". *Tetrahedron Letter*, vol. 6, pp.4857–4864.

Clarkson, C., V. J. Maharaj, N. R. Crouch, O. M. Grace, P. Pillay, M. G. Matsabisa, N. Bhagwandin, P. J. Smith and P. I. Folb. (2004). "In *Vitro* Antiplasmodial Activity of Medicinal Plants Native to or Naturalized in South Africa". *Journal of Ethnopharmacology*, vol.92, 177-191.

Deciga-Campos M, I. Rivero-Cruz, M. Arriaga-Alba, G. Castaneda-Corral, G. E. Angeles-Lopez (2007). "Acute Toxicity and Mutagenic Activity of Mexican Plants Used in Traditional Medicine". *J Ethnopharmacol*., vol.110, pp. 334-342.

Galeffi, C., E. M. delle Monache, C. G. Casinovi, and G. B. M. Bettòlo. (1969). "A New Quinone from the Heartwood of *Mansonia altissima* Chev: Mansonone L". *Journal of Integrative Agriculture,* vol. 10, pp.3583–3584.

Maisarah, A. M., B. N. Amria, R. Asmah and O. Fauziah. (2013). "Antioxidant Analysis of Different Parts of Corica papaya". *IFRJ*, vol.20 (3), pp. 1043-1048

Olowa L.F. and M. N. Olga. (2013). "Brine Shrimp Lethality Assay of the Ethanolic Extract of Three Selected Species of Medicinal Plants from Iligan City, Philippines". *Int Res J Biol Sci*., vol. 2, pp.4-77.

Selvamohan, T., V.Ramadas and S. S. S. Kishore. (2012). "Antimicrobial Activity of Selected Medicinal Plants against Some Selected Human Pathogenic Bacteria".*Adv. Appl. Sci. Res.,* vol. 3 (5), pp.3374-3381.

Wilfrid K., D-D. Agboddiogbe, S.I. Albérick, TiTo, Gérard Adjakpahoun, F. Messan., and P.H. Dansou. (2022). "Antioxidant Properties of *Senna siamea* and Effects on Sports Performance in Wistar Rats". *International Journal of Biological and Chemical Science*, vol.16(3), pp.1193-1203.