



## ORIGINAL ARTICLE

# Total phenolic and flavonoid contents, antioxidant, antidiabetic and antiplasmodial activities of *Garcinia forbesii* King: A correlation study



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## KEYWORDS

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**Abstract** *Garcinia forbesii* King belongs to Clusiaceae is a source of secondary metabolites especially xanthenes with various biological activities. *G. forbesii* King is also known for its empirical use for malaria and diabetes. This study investigated the total phenolic and flavonoid contents, *in vitro* antioxidant, antidiabetic and antiplasmodial activities of four extracts attained from the stem bark of *G. forbesii* King. The total phenolic and flavonoid contents were determined by spectrophotometric methods and antioxidant activity was evaluated by DPPH, ABTS, FRAP assays. *In vitro* antidiabetic activity was assessed by  $\alpha$ -glucosidase and  $\alpha$ -amylase assays and antiplasmodial activity was studied against chloroquine sensitive *Plasmodium falciparum* strain 3D7. The highest value of total phenolic ( $187.37 \pm 0.06$  mg GAE/g) and flavonoid ( $35.97 \pm 0.02$  mg QE/g) contents were recorded in *n*-hexane and methanolic extracts. *n*-Hexane extract showed the highest DPPH activity with  $IC_{50}$  of  $8.12 \pm 0.02$   $\mu$ g/mL. Ethyl acetate extract exhibited better scavenging ability for ABTS with  $IC_{50}$  of  $3.88 \pm 0.04$   $\mu$ g/mL. The FRAP assay showed better activity in methanol extract with an inhibition value of  $73.68 \pm 3.66$   $\mu$ M  $Fe^{2+}$ /g. The strong inhibition against  $\alpha$ -glucosidase and  $\alpha$ -amylase were displayed by dichloromethane extract with  $IC_{50}$  of  $35.13 \pm 2.01$   $\mu$ g/mL and  $4.83 \pm 0.20$   $\mu$ g/mL. *n*-Hexane and methanol extracts showed significant antiplasmodial

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activity with  $IC_{50}$  of  $0.23 \pm 0.01 \mu\text{g/mL}$  and  $0.73 \pm 0.01 \mu\text{g/mL}$ , respectively. The correlation analysis indicated a positive relationship of total phenolic and flavonoid contents with antiplasmodial activity. The results revealed that *n*-hexane and methanol extracts could be used as a potential natural antiplasmodial, while dichloromethane extract is a promising natural antidiabetic.

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## 1. Introduction

*Garcinia forbesii* King is a small to medium-sized perennial tree belongs to the family Clusiaceae and is native to Indonesia (Sumatera, Kalimantan) and Malaysia (Perry and Metzger, 1980; Aravind et al., 2016, Lim, 2012). *G. forbesii* King is known as Red Mangosteen, Forest Mangosteen, or Rose Kandis and locally referred as Kandis, Mundar (Sumatera), Bunoh, Itan, Bua' Tetoh Telato, Aiti Kitung (Kalimantan) in Indonesia and as Kandis, Assam Roi, Babata in Malaysia (Lim, 2012; Sutomo et al., 2020). It is an evergreen tropical plant adapted to a wet, hot, humid environment mainly found in tropical rain forests (Lim, 2012). The white and sour-sweet fruit of this plant is edible, and the rind has been used as flavouring for various types of cuisines (Lim, 2012; Noor and Senin, 2017).

Phytochemical studies indicate that *Garcinia* species including *G. forbesii* King are rich of secondary metabolite especially xanthenes. Xanthenes are well known for their various bioactivities such as anti-inflammatory (Zhang et al., 2014), antiviral, antimalarial (Elfita et al., 2009), antidiabetic (Phukhatmuen et al., 2020), antifungal (Gopalakrishnan et al., 1997) and anticancer (Sukandar et al., 2020). Furthermore, xanthenes are effective against free radicals due to the presence of hydroxy groups in its skeleton (Taher et al., 2012). Harrison and co-worker reported the isolation of a chromenoxanthone namely forbesanthone along with pyranojacareubin and 1,3,7-trihydroxy-2-(3-methylbut-2-enyl)-xanthone from the branches of *G. forbesii* King (Harrison et al., 1993). Forbesione, a caged xanthone, was also obtained from *G. forbesii* King (Leong et al., 1996). Ethnobotanically *G. forbesii* King has been empirically used by local people in East Part of Indonesia as a folk medicine to treat malaria and diabetes.

Though *G. forbesii* King shows beneficial potency in traditional medication, there is a void in the relationship of phytochemical profile and its *in vitro* biological activities. Considering these aspects, here we report a correlation study on total phenolic and flavonoid contents, antioxidant activity using DPPH, ABTS, and FRAP assays,  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory and antiplasmodial activities of *G. forbesii* King using four solvents to verify their medication potency. This study, to the best of our knowledge, is the first report focusing on the correlation of phenolic and flavonoid contents with antioxidant, antidiabetic and antiplasmodial activities of *G. forbesii* King stem bark.

## 2. Material and methods

### 2.1. Plant material

The stem bark of *G. forbesii* King was collected from the Somaetek forest in North Halmahera, Indonesia ( $1^{\circ}25'22''$  LU  $127^{\circ}46'59''$  E). This plant was identified and the specimen (VII.G.237a) was stored in the Bogor Botanical Gardens.

### 2.2. Preparation of extracts

The bark of *G. forbesii* King was dried at  $50^{\circ}\text{C}$  and crushed into powder (60–100 mesh) using a milling machine. A total of 15 g of the powder was weighed and extracted by maceration (150 mL) with four solvents: *n*-hexane, dichloromethane, ethyl acetate and methanol. The obtained extracts were then filtered and evaporated under reduced pressure ( $40$ – $60^{\circ}\text{C}$ ) to give dry extracts.

### 2.3. Total phenolic and flavonoid contents

The Folin-Ciocalteu method was employed for total phenolic content determination following previously reported paper (Badrulhisham et al., 2020) with slight modifications using gallic acid as standard (0–200 mg/L). Briefly, 0.5 mL of the extracts in methanol (1000 ppm) was mixed with 2.5 mL of 10% aqueous Folin-Ciocalteu solution, stirred and left for 5 min. A 2.0 mL of 5% aqueous  $\text{Na}_2\text{CO}_3$  solution was then added. The mixture was further incubated at  $40^{\circ}\text{C}$  for one hour and the absorbance was measured at a wavelength of 765 nm by using a UV-Vis spectrophotometer (Genesys UV-Vis Spectrophotometer, Thermo Fisher Scientific, Madison, WI, USA) with methanol as the blank.

The aluminum chloride method was used for total flavonoid content (Armania et al., 2013) evaluation using quercetin as standard (0–50 mg/L) in methanol. A total of 0.5 mL of each extract (100–1000 ppm) was mixed with 0.5 mL of 2%  $\text{AlCl}_3$  (in methanol) followed by incubation for one hour and the absorbance was measured at 415 nm using UV-Vis spectrophotometer (Genesys UV-Vis Spectrophotometer, Thermo Fisher Scientific, Madison, WI, USA) with methanol as the blank.

### 2.4. DPPH radical scavenging assay

The DPPH antioxidant activity of extracts was determined following the reported papers (Brand-Williams et al., 1995; Dudonné et al., 2009). A 3.0 mL of  $6 \times 10^{-5}$  M DPPH was added to 100  $\mu\text{L}$  of extracts (maximum dissolved concentration) in methanol. After 20 min of incubation at  $37^{\circ}\text{C}$ , the absorbance of the mixture was assessed at a wavelength of 517 nm (As). The absorbance of the blank was also measured at the same wavelength (Ab). This experiment was carried out in triplo using quercetin and gallic acid as controls. The radical inhibitory activity was calculated using the equation  $[(\text{Ab}-\text{As}) / \text{Ab}] \times 100\%$ .

### 2.5. ABTS radical scavenging assay

The ABTS radical scavenging activity was evaluated based on the previous papers (Armania et al., 2013; Arteaga-Crespo

et al., 2020). The ABTS solution was prepared from 5.0 mL of 7 mM ABTS and 88 mL of potassium peroxydisulfate ( $K_2S_2O_8$ ). The mixture was left to stand for 12–16 h at room temperature and a dark blue solution was formed. A 250 mL of 99.5% ethanol was then added to the solution, which provided an absorbance of  $0.7 \pm 0.02$  at 734 nm. This was referred as working solution. A total of 1.0 mL of working solution and 10  $\mu$ L of the extracts (maximum dissolved concentration) in methanol was shaken for 10 s and incubated at 30 °C for 4 min. The absorbance of the mixture ( $A_s$ ) was measured at a wavelength of 734 nm with ethanol as a blank ( $A_b$ ). Quercetin and gallic acid were used as controls. The ABTS inhibition was calculated by  $[(A_b - A_s) / A_b] \times 100$ .

#### 2.6. Ferric reducing-antioxidant power (FRAP) assay

The iron reduction power of *G. forbesii* King extracts was evaluated by a method of Dudonné et al. (2009) with a slight modification. This assay was conducted based on the reduction of a colorless iron complex of  $Fe^{3+}$ -tripyridyltriazine to a blue complex of  $Fe^{2+}$ -tripyridyltriazine. The FRAP reagent was mixed with freshly prepared 300 mM acetate buffer, 40 mM HCl, 10 mM TPTZ and 20 mM  $FeCl_3 \cdot 6H_2O$  at a ratio of 10:1:1 (acetate buffer:TPTZ: $FeCl_3 \cdot 6H_2O$ ). The standard curves were made using various concentrations of  $FeSO_4 \cdot 7H_2O$  and all solutions were prepared in a fresh condition. About 100  $\mu$ L of sample solution, 900  $\mu$ L of distilled water, and 2 mL of FRAP reagent were mixed and incubated at 37 °C for 30 min and the absorbance was measured at 593 nm. The blank sample measurement was carried out using acetate buffer. The difference between the absorbance of the samples and the absorbance of the blank was calculated and utilized to obtain the FRAP value and extracts' reduction capacity. The FRAP value was expressed as  $\mu$ M  $Fe^{2+}$ /g ((FRAP Value of sample ( $\mu$ M)) = abs (sample)  $\times$  FRAP value of std ( $\mu$ M) / abs (std)). Ascorbic acid was used as control and all measurements were carried out in triplicate.

#### 2.7. Rat intestinal $\alpha$ -glucosidase inhibitory activity

The inhibitory activity of rat intestinal  $\alpha$ -glucosidase was determined according to a modified method (Worawalai et al., 2012). This technique was carried out by grouping four mixtures into four different categories: (1) blank enzyme reaction, (2) enzyme reaction, (3) blank samples reaction, (4) samples reaction. The mixture of (1) contained DMSO (10  $\mu$ L), sodium phosphate buffer (50  $\mu$ L), glucose kit (80  $\mu$ L). The enzyme reaction group (2) consisted of mixture (1) with the addition of maltose or sucrose substrates (20  $\mu$ L). The positive control of acarbose was similarly prepared as (2). The group (3) was loaded with identical mixture of (1) with samples (10  $\mu$ L) replacing the DMSO. The last mixture of (4) composed of (3) and substrates maltose or sucrose (20  $\mu$ L). The well plates were then incubated for 10 min (maltose) or 40 min (sucrose). A microplate reader was used to measure the absorbance at 520 nm (BioTek ELx800TM, BioTek Instruments, Inc., Winooski, VT, USA).

#### 2.8. $\alpha$ -Amylase inhibitory activity

The  $\alpha$ -amylase activity was evaluated using a method modified by Unuofin et al., 2018. A 5.0 mg of  $\alpha$ -amylase (Porcine pan-

creatic  $\alpha$ -amylase) in 1.0 mL 0.1 M phosphate buffer pH 6.9 was added to a solution of 10 mg samples in 1.0 mL DMSO. The samples (20  $\mu$ L) were mixed with freshly prepared potato substrate (50  $\mu$ L) and 30  $\mu$ L of phosphate buffer (pH 6.9). The substrate was obtained by heating potato starch (100 mg) in 5.0 mL 0.1 M phosphate buffer for 5 min and cooled at room temperature. After a 5-minute pre-incubation period at room temperature, 20  $\mu$ L of  $\alpha$ -amylase was then added. Following that, the solution was incubated at 37 °C for 15 min and a color change was noticed by adding 50  $\mu$ L of 1 M HCl and 50  $\mu$ L of 10% iodine solution. A UV-Vis spectrophotometer (Genesys UV-Vis Spectrophotometer, Thermo Fisher Scientific, Madison, WI, USA) was used for absorbance measurement at a wavelength of 650 nm.

#### 2.9. Antiplasmodial activity

The antiplasmodial activity of the extracts was judged by a method of Budimulja et al. (1997) using chloroquine sensitive *Plasmodium falciparum* strain 3D7. Samples were made at concentrations of 1000, 100, 10, 1 and 0.1  $\mu$ g/mL. The parasites used in this test were synchronous (Ring stage) with  $\pm$  1% parasitemia. A 2.0  $\mu$ L of the test solution with various concentrations was pipetted to 96 wells followed by 198  $\mu$ L of parasites. The well was further placed in a chamber with  $O_2$  5%,  $CO_2$  5%, and  $N_2$  90% atmosphere. The chamber was then incubated at 37 °C for 48 h and subsequently the culture was harvested, and a thin blood layer was made with 20% Giemsa staining. Data analysis was carried on the blood smear that has been made by counting the number of infected erythrocytes in every 1000 normal erythrocytes under a microscope. The data was further used to determine the percent growth and inhibition.

#### 2.10. Statistical analysis

The statistical analysis was carried out with IBM 23 statistical software to determine total phenolic and flavonoid contents, antioxidant activities and antidiabetic activities. This study was carried out in thrice to determine the mean value (mean SD). A linear regression equation was used to determine the percentage of  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibition of each extract's concentration. Meanwhile, probity analysis was used to determine the proportion of growth inhibition on *P. falciparum*.

### 3. RESULTS AND discussion

#### 3.1. Total phenolics and total flavonoids contents

In this study, the total phenolic and flavonoid contents of *n*-hexane, dichloromethane, ethyl acetate and methanol extracts from the bark of *G. forbesii* King were tested. The results of total phenolic content were expressed as gallic acid equivalents using the equation of a standard curve  $y = 0.0042x + 0.0234$ ,  $R^2 = 0.9901$ , as shown in Fig. 1a. The *n*-hexane extract showed the highest phenolic content ( $187.37 \pm 0.06$  mg GAE/g dry extract). Ramirez and co-workers (2019) found the same profile of *n*-hexane extract with high phenolics from the epicarp and leaves of *G. madruno*. In contrast, methanol extract, as shown in Table 1, had the lowest phenolic contents ( $73.40 \pm 0.11$  mg GAE/g dry extract). This result indicated that phenolic compounds of *G. forbesii* King were accumulated

in nonpolar and semipolar extracts, and less found in polar extract. This finding was supported by the isolation of five xanthenes from dichloromethane extract, including lichexanthone, subelliptenone H, 12b-hydroxy-des-D-garcigerrin A, garcini-xanthone B and garcigerin A (Wairata et al., 2021). The flavonoid contents were expressed as quercetin equivalent using the equation  $y = 0.0337x$ ;  $R^2 = 0.9973$ , as shown in Fig. 1b. The methanol extract displayed the highest flavonoids ( $35.97 \pm 0.02$  mg EQ/g dry extract) while the *n*-hexane extract was the lowest ( $6.60 \pm 0.14$  mg EQ/g dry extract). It was noticed that flavonoid compounds were accumulated in polar solvents and lower in nonpolar solvents (Arteaga-Crespo et al., 2020; Barbouchi et al., 2020).

### 3.2. Antioxidant activity

#### 3.2.1. Radical-scavenging activity (DPPH and ABTS assays)

The antioxidant assay was carried out by *in vitro* DPPH method. This method has been employed for the evaluation of free radical scavenging activity of several natural product extracts (Terpinc et al., 2012; Mbaoji and Nweze, 2020). Table 2 showed the free radical scavenger activity based on DPPH method at a concentration of  $159.7 \mu\text{g/mL}$  with quercetin and gallic acid as controls. The *n*-hexane extract showed stronger DPPH radical-scavenging activity with  $\text{IC}_{50}$  of  $8.12 \pm 0.02 \mu\text{g/mL}$ . This result was positively correlated with phenolic contents of *n*-hexane extract.

Furthermore, the ABTS which stabilizes the free radicals through proton donors and provides lipophilic and hydrophilic compounds (Chohra et al., 2020; Aguirre-Becerra et al., 2020) was also utilized for the evaluation of free radical scavenging activity of extracts using quercetin and gallic acid as standards. The results, as shown in Table 2, indicated that the ethyl acetate extract exhibited better inhibitory activity with  $\text{IC}_{50}$  of  $3.88 \pm 0.04 \mu\text{g/mL}$ . The ABTS activity of ethyl acetate and methanol extracts was higher compared to dichloromethane and *n*-hexane extracts. This trend was also reported by Floegel and co-workers (2011).

#### 3.2.2. Ferric reducing-antioxidant power (FRAP) assay

The antioxidant potential of all extracts was also evaluated by using the FRAP assay (Tai et al., 2011). The iron reduction properties are frequently used as an indicator of electron activity which is an important part of antioxidant activity (Prior

**Table 1** TPC and TFC Values of *G. forbesii* Extract.

<i>G. forbesii</i> Extracts	TPC (mg GAE/g dry extract) <sup>a</sup>	TFC (mg QE/g dry extract) <sup>b</sup>
<i>n</i> -hexane	$187.37 \pm 0.06$	$6.60 \pm 0.14$
Dichloromethane	$127.84 \pm 0.05$	$24.25 \pm 0.12$
Ethyl Acetate	$116.65 \pm 0.06$	$34.21 \pm 0.01$
Methanol	$73.40 \pm 0.11$	$35.97 \pm 0.02$

<sup>a</sup> mg GAE/g dry extract gallic acid equivalents per g dry weight.

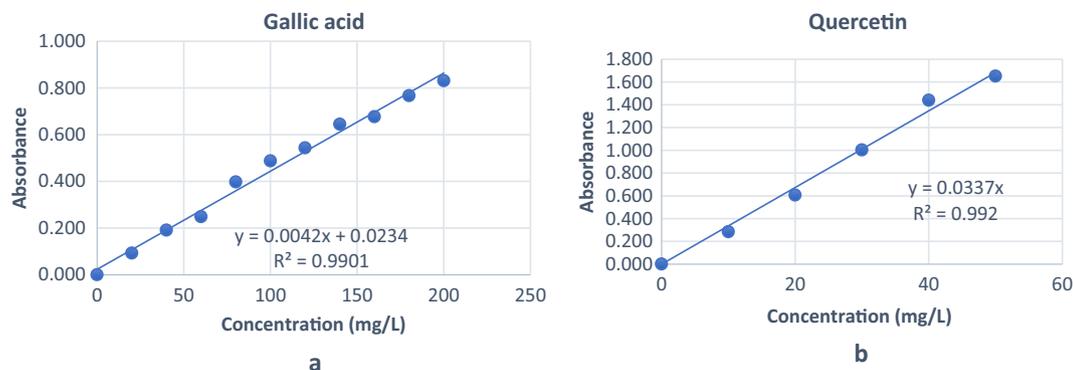
<sup>b</sup> mg QE/g dry extract quercetin equivalents per g dry weight.

et al., 2005). Table 2 displayed that the reduction power of methanol extract was the highest ( $73.68 \pm 3.66 \mu\text{M Fe}^{2+}/\text{g}$ ) compared to other extracts and ascorbic acid as standard ( $30.62 \pm 0.27 \mu\text{M Fe}^{2+}/\text{g}$ ). The reduction properties of all extracts indicated that increasing polarity may lead to an increase in the reduction power. The antioxidant potential of *G. forbesii* King extracts was verified by FRAP assay.

#### 3.3. $\alpha$ -Glucosidase and $\alpha$ -amylase inhibitory activities

The enzyme inhibitory activity for all extracts was assessed using  $\alpha$ -glucosidase and  $\alpha$ -amylase. The  $\alpha$ -glucosidase and  $\alpha$ -amylase are key enzymes involved in carbohydrate metabolism.  $\alpha$ -Amylase degrades complex carbohydrates into oligosaccharides and disaccharides which further degrade into monosaccharides by  $\alpha$ -glucosidase. The liberated glucose is then absorbed by the intestine and causes postprandial hyperglycemia. The inhibition of this process reduces the postprandial glucose levels by slowing the hydrolysis and absorption of carbohydrates in the blood which is useful in the treatment of type 2 diabetes (Shinde et al., 2008).

The  $\alpha$ -glucosidase inhibitory activity of extracts from *G. forbesii* King was observed using rat intestinal enzymes with sucrose and maltose substrates.  $\alpha$ -Glucosidase breaks sucrose into glucose and fructose while maltose becomes two glucoses (Kadouh et al., 2016; Unuofin et al., 2018). Table 3 showed that the inhibition percentage and  $\text{IC}_{50}$  value of dichloromethane extract using sucrose substrate were  $86.68 \pm 0.05\%$  and  $35.13 \pm 2.01 \mu\text{g/mL}$  at a concentration of  $312.5 \mu\text{g/mL}$ . This value was the highest compared to other extracts. The *n*-hexane extract produced lowest inhibition in sucrose with



**Fig. 1** Standard curves for gallic acid (a) and quercetin (b).

**Table 2** Antioxidant activities of *G. forbesii* extracts.

<i>G. forbesii</i> Extract	Antioxidant activity				
	FRAP	DPPH		ABTS	
	$\mu\text{M Fe}^{2+}$	% Inhibition <sup>a</sup>	IC <sub>50</sub> ( $\mu\text{g/mL}$ )	% Inhibition <sup>b</sup>	IC <sub>50</sub> ( $\mu\text{g/mL}$ )
n-hexane	24.79 $\pm$ 1.16	90.05 $\pm$ 0.02	8.12 $\pm$ 0.02	83.93 $\pm$ 0.01	11.38 $\pm$ 0.06
Dichloromethane	68.63 $\pm$ 2.01	93.95 $\pm$ 0.05	14.40 $\pm$ 0.04	90.64 $\pm$ 0.04	10.28 $\pm$ 0.03
Ethyl Acetate	69.14 $\pm$ 1.53	91.82 $\pm$ 0.08	10.80 $\pm$ 0.01	97.53 $\pm$ 0.01	3.88 $\pm$ 0.04
Methanol	73.68 $\pm$ 3.66	88.71 $\pm$ 0.05	10.80 $\pm$ 0.02	90.73 $\pm$ 0.03	3.97 $\pm$ 0.02
Ascorbic acid	30.62 $\pm$ 0.27	Nt	Nt	Nt	Nt
Quercetin (C)	Nt	96.34 $\pm$ 0.01	1.24 $\pm$ 0.01	97.58 $\pm$ 0.01	0.05 $\pm$ 0.01
Gallic acid (C)	Nt	97.21 $\pm$ 0.01	0.51 $\pm$ 0.01	96.30 $\pm$ 0.01	0.12 $\pm$ 0.01

Nt = Not tested.

<sup>a</sup> = tested at concentration of 159.7  $\mu\text{g/mL}$ .

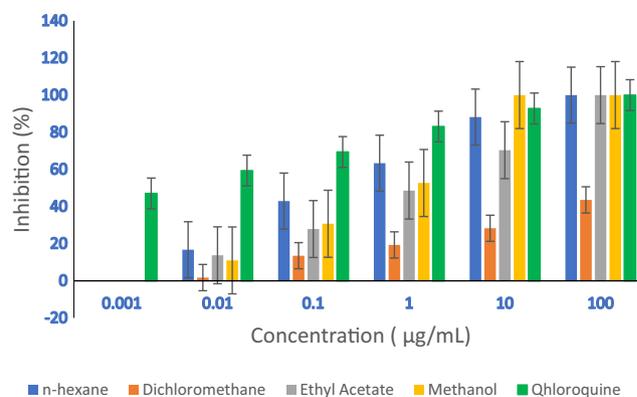
<sup>b</sup> = tested at concentration of 99  $\mu\text{g/mL}$ .

63.14  $\pm$  0.02% of inhibition and IC<sub>50</sub> of 60.87  $\pm$  1.06  $\mu\text{g/mL}$ . In contrast, the *n*-hexane extract displayed the highest activity using maltose with a percent inhibition and IC<sub>50</sub> value of 86.32  $\pm$  0.02% and 38.94  $\pm$  1.45  $\mu\text{g/mL}$ . The methanol extract was the least active extract in maltose with 64.14  $\pm$  0.01% of inhibition and IC<sub>50</sub> of 29.89  $\pm$  1.54  $\mu\text{g/mL}$ . These data indicated that nonpolar extracts were very active for  $\alpha$ -glucosidase inhibition compared to others.

$\alpha$ -Amylase is an enzyme responsible for starch processing into glucose (Venkatachalam et al., 2020). Therefore, the inhibitory activity of the extracts from *G. forbesii* King was also tested against this enzyme. The dichloromethane extract showed a strong inhibition activity against  $\alpha$ -amylase with IC<sub>50</sub> value of 4.83  $\pm$  0.20  $\mu\text{g/mL}$  at a concentration of 99.01  $\mu\text{g/mL}$  (Table 3). The lowest activity was shown by methanol extract with IC<sub>50</sub> of 16.47  $\pm$  0.51  $\mu\text{g/mL}$ . The standard acarbose had an IC<sub>50</sub> value of 2.61  $\pm$  0.32  $\mu\text{g/mL}$ . The  $\alpha$ -amylase activity evaluation confirmed the profile of nonpolar and semipolar extracts in *G. forbesii* King.

### 3.4. Antiplasmodial activity

The *in vitro* antiplasmodial assay of *G. forbesii* King stem bark extracts were performed against *P. falciparum* strain 3D7. The results, as presented in Table 4 and Fig. 2, revealed that *n*-hexane, ethyl acetate, and methanol extracts showed promising



**Fig. 2** Percentage of antiplasmodial inhibition activities of *G. forbesii* extracts.

activity at a 100  $\mu\text{g/mL}$  concentration compared to chloroquine standard. The methanol extract produced the highest activity at a concentration of 10  $\mu\text{g/mL}$  compared to other extracts and chloroquine. Furthermore, at a concentration of 1  $\mu\text{g/mL}$ , the inhibition percentage of *n*-hexane extract was 63.32%. The IC<sub>50</sub> values of the *n*-hexane and methanol extracts were of 0.23  $\pm$  0.01  $\mu\text{g/mL}$  and 0.73  $\pm$  0.01  $\mu\text{g/mL}$  close to the chloroquine standard (IC<sub>50</sub> 0.002  $\pm$  0.01  $\mu\text{g/mL}$ ). The IC<sub>50</sub> values in the range of 10 to  $\leq$  10  $\mu\text{g/mL}$  were expressed as very active.

**Table 3** The *in vitro* anti diabetic of *G. forbesii* extracts.

<i>G. forbesii</i> Extract	$\alpha$ -glucosidase				$\alpha$ -amylase	
	Rat intestinal					
	% Inhibition	Sucrose IC <sub>50</sub> ( $\mu\text{g/mL}$ )	% Inhibition	Maltose IC <sub>50</sub> ( $\mu\text{g/mL}$ )	% Inhibition	Starch IC <sub>50</sub> ( $\mu\text{g/mL}$ )
n-hexane	63.14 $\pm$ 0.02	60.87 $\pm$ 1.06	86.32 $\pm$ 0.02	38.94 $\pm$ 1.45	69.41 $\pm$ 0.05	8.12 $\pm$ 0.50
Dichloromethane	86.68 $\pm$ 0.05	35.13 $\pm$ 2.01	94.89 $\pm$ 0.05	34.24 $\pm$ 1.60	84.27 $\pm$ 0.01	4.83 $\pm$ 0.20
Ethyl Acetate	68.26 $\pm$ 0.02	55.47 $\pm$ 1.50	95.50 $\pm$ 0.01	24.88 $\pm$ 1.27	74.62 $\pm$ 0.04	7.91 $\pm$ 0.35
Methanol	70.33 $\pm$ 0.01	54.27 $\pm$ 1.20	91.60 $\pm$ 0.01	29.89 $\pm$ 1.54	65.96 $\pm$ 0.02	16.47 $\pm$ 0.51
Acarbose	96.76 $\pm$ 0.01	2.97 $\pm$ 0.02	97.60 $\pm$ 0.01	2.21 $\pm$ 0.27	94.11 $\pm$ 0.02	2.61 $\pm$ 0.32

**Table 4** The *in vitro* antiplasmodial of *G. forbesii* extracts.

<i>G. forbesii</i> Extracts	IC <sub>50</sub> (µg/mL)
n-hexane	0.23 ± 0.01
Dichloromethane	> 100 ± 0.50
Ethyl Acetate	1.11 ± 0.10
Methanol	0.73 ± 0.01
Chloroquine	0.002 ± 0.01

Therefore, the extracts of *n*-hexane and methanol were active (IC<sub>50</sub> value ≥ 10 µg/mL) (Kainama et al., 2019). The results indicated that the extracts of *G. forbesii* King are potential therapeutics against *P. falciparum* 3D7 strain (Lulan et al., 2020).

### 3.5. Correlation analysis

This study correlates the total phenolic and flavonoid contents, antioxidant, antidiabetics, and antiplasmodial activities of *G. forbesii* Kings extracts. The relationship was calculated by using Kendall's Tau correlation. Hunter and co-workers (2021) reported the use of Kendall's Tau calculation in the correlation of Manuka-type honeys with their bioactivities. The total phenolics negatively correlated with total flavonoids ( $r = -1,000, p < 0.01$ ) means that the higher the total phenolic content, the lower the total flavonoid content. In general, total phenolics and flavonoids play an important role in the antioxidant activity acting as a chain breaker, free radical scavenger and electron donor (Kainama et al., 2020). Table 5 shows a strong positive correlation between DPPH vs total phenolics and DPPH vs total flavonoids with  $r = 0.718$  ( $p < 0.005$ ). A weak correlation was observed between ABTS vs total phenolics and ABTS vs total flavonoids with  $r = 0.174$  ( $p < 0.05$ ), but ABTS had a strong correlation with DPPH ( $r = 0.718, p < 0.05$ ). The total phenolics and FRAP were negatively fierce ( $r = -1,000, p < 0.01$ ) which shows an inverse relationship between phenolics and Fe<sup>3+</sup> reduction activity. The higher the phenolic, the lower the reduction activity. In contrast, a positive correlation was occurred for total flavonoids vs FRAP ( $r = 1.000, p < 0.01$ ). This indicated the flavonoid

compounds contained in the extracts were directly proportional to the reduction power. Furthermore, the ABTS was strongly correlated with α-glucosidase inhibitory activity (sucrose  $r = 1.000, p < 0.05$ ; maltose  $r = 1.000, p < 0.01$ ). In addition, the α-amylase inhibitory activity was also showed a relationship with ABTS ( $r = 1.000, p < 0.05$ ). This data concluded that there is a correlation between antidiabetic and antioxidant activities. The lipophilic and hydrophobic compounds are active in ABTS and as antidiabetics (Huang et al., 2020).

## 4. Conclusion

This study reports on correlation of total phenolics and flavonoids, *in vitro* antioxidant, antidiabetic and antiplasmodial activities of *G. forbesii* King stem bark using *n*-hexane, dichloromethane, ethyl acetate and methanol. The use of four different solvents was purposely for designing the *in vitro* assays which yielded a various class of phytochemicals from nonpolar, semipolar, and polar. The use of organic solvents in the *in vitro* assay is acceptable due to their volatility and they have been completely removed from the extract. A comprehensive study revealed that *G. forbesii* King is a promising resource of potential bioactive metabolites for medicinal and pharmaceutical purposes. The *n*-hexane and methanolic extracts of *G. forbesii* King stem bark recorded with higher content of phenolics and flavonoids. These compounds possess strong positive correlation with *in vitro* antioxidant results. A correlation was also noted between antidiabetic and antioxidant activities. This study noticed that the nonpolar extract correlated with antidiabetic activity while the polar extract showed a relationship with antiplasmodial activity. Investigations are needed to understand the mechanism of action of purified compounds composed the extracts based on the results of this study.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Table 5** Kendall's tau-b correlation coefficient of total phenolic activity, total flavonoids, antioxidants, and anti diabetic.

	TPC <sup>a</sup>	TFC <sup>b</sup>	DPPH <sup>c</sup>	ABTS <sup>c</sup>	FRAP <sup>c</sup>	Sucrose <sup>d</sup>	Maltose <sup>d</sup>	Strach <sup>d</sup>
TPC	1							
TFC	-1.000**	1						
DPPH	0.718*	0.718*	1					
ABTS	0.174*	0.174*	0.718*	1				
FRAP	-1.000**	1.000**	0.718*	0.174*	1			
Sucrose	0.497*	0.497*	0.071*	1.000*	0.497*	1		
Maltose	0.174*	0.174*	0.718*	1.000**	0.174*	1.000*	1	
Strach	0.497*	0.497*	0.279*	1.000*	0.497*	0.497*	1.000*	1

\* Correlation is significant at the 0.05 level.

\*\* Correlation is significant at the 0.01 level.

<sup>a</sup> Total phenolic content.

<sup>b</sup> Total Flavonoids content.

<sup>c</sup> Coefficient of antioxidant.

<sup>d</sup> Coefficient of antidiabetic.

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