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In-vivo therapeutic efficacy and phytochemical investigation of three commonly used plants for malaria treatment by the Hausa community in Kano, Nigeria

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Abstract
It is estimated that about 97% of Nigeria population are at menace of malaria infection, with Kano state having the highest prevalence of infection at the moment. Yet, substantial number of the infected populace used folk treatment as substitute for the orthodox medicine. The present study investigated *in-vivo* antimalaria activities and phytochemical constituent of the back extracts of *Boswillia dalzielii, Diospyros mespiliformis and Ficus sycomorus* which are the three most common plants used in Kano for folklore treatment of malaria infection. Fifty-five (55) pure strains of Adult Swiss albino mice inoculated with chloroquine resistant NK65 lineage of *Plasmodium berghei* were used to evaluate the antimalarial activity of the back extract of the three selected plants. The infected animals were randomized into 11 groups of 5 animals. Nine groups were treated with methanol extract of the three plant species at concentration 200mg/kg, 400mg/kg and 800mg/kg body weight. The remaining two groups were treated with either water (Negative control) or chloroquine (positive control). The result revealed that though all the plant investigated possess antimalaria properties, their chemotherapeutic activity against malaria infection is dose dependent. At the dose of 800 mg/kg, back extract of *F. sycomorus* and *D. mespiliformis* had higher antimalaria activities with suppression percentage of 65.79 and 53.25, respectively. The presence or absence of secondary metabolites varies among the plants and depends on the type of solvent used for extraction. Alkaloids, flavonoids, glycosides, triterpenoids and phenols were present in all the three species, but mostly in different extracting solvent. The results pointed to better potency of *D. mespiliformis* and *F. sycomorus* in treating malaria infection compared to *B. dalzielii* and can be a good source for the industrial manufacturing of antimalarial drugs.

Keywords: antimalaria; drugs; *B. dalzielii*; *F. sycomorus*; *D. mespiliformis*; phytochemical

Introduction
Over the years, malaria, an *Anopheles* mosquito borne parasitic disease has persistently be one of the leading causes of morbidity and mortality despite that it is a curable (Kigondu *et al*. 2011; Bankole *et al*. 2016) [17, 10]. At present, 33,000 cases of malaria per 100,000 people has been noted with 110, 000 mortality recorded. The global spread of malaria owing to the inadequacy of vector control programme and increasing resistance to known antimalarial drugs has resulted into a huge burden of negative consequences on general well-being of many people (Ettebong *et al*. 2012) [13]. The disease is noted to account for 44.7 million disability adjusted life years (DALYs), millions of death cases (Ishola *et al*. 2014; WHO, 2015; Oladeji *et al*. 2020) [16, 38, 26] and continue to pose a grave menace to human health in 97 countries and territories of tropical and subtropical regions (Adebayo *et al*. 2017). Although malaria case is well known in Caribbean, Asia, and South American, the surge in Africa is alarming as approximately 19 million infections and nearly 17 million deaths have been reported (WHO, 2015; Oladeji *et al*. 2020) [38, 26], thus accounted for 89% of the global cases. Majority of Africa infection cases domicile in 13 countries, and over half in Nigeria, Congo, Ethiopia, Tanzania and Kenya (WHO, 2008a) [37].
In Nigeria, malaria remains a major health and economic burden to teeming population as almost 51 million infection and 207, 000 deaths are published annually (FMH, 2012). It is estimated that about 97% of Nigeria population are facing menace of malaria infection (WHO. 2014), whereas at present, the disease accounted for about 60 % of outpatient appointments in hospitals (Dawaki *et al*. 2016). Occurrence and transmission of malaria among citizens across Nigeria states is uneven, thus making treatment approach inconsistent. For example, malaria occurrence and transmission occur all year round in southern states of Nigeria, but highly

seasonal in the north state (Adebayo and Krettli, 2011) [3]. However, a recent report from the prevalence study conducted across Nigeria shows that the North West and North Central zones have high incidence of malaria cases (MIS, 2015). According to Kano state ministry of health, Kano which is the most populated among the northern state had the highest prevalence of malaria in year 2016 and 2017 throughout the country.

Regardless of the seasonality in occurrence of malaria, poorer and more rural populations are at greater menace of infections, likely due to poverty and inability to access orthodox health system. In addition to the inability of most rural dweller to access available malaria drugs, the incessant resistance of *Plasmodium falciparum*; the deadliest form of all malaria parasites (Ngarivhume *et al*. 2015) to antimalarial drugs poses a serious risk to malaria control programs. For example, a nationwide survey by Nigeria Federal Ministry of Health on efficacy of some malarial drugs, particularly chloroquine and (CQ) and sulphadoxine-pyrimethamine (SP) showed that their therapeutic effect is becoming less viable (FMH, 2004). At the background of the present-day scenario, it is very important to find all-inclusive medicines with novel mechanisms of action for the treatment of certain diseases such as malaria, which do not need prolonged treatment and may be cured by the use of herbal products.

The use of medicinal plant in treatment of malaria around the world is not new as most these plants are known to be excellent source of phytochemicals such as alkaloids, tannins, flavonoids, and phenolic compounds (Kigondu *et al*. 2011; Bankole *et al*. 2016) [17, 10] that aids human body in maintaining active defense system and general wellbeing. They are considered to be affordable, less toxic and source of new phytochemicals that possibly could be industrialized as useful medications for the treatment of malaria and other infectious diseases (Adebayo and Krettli, 2011) [3]. However, the approach employed in selecting plant to be used for malaria treatment in the past is very arduous and often yield fewer valuable results. For instance, in the past, about 600 plant species from 126 families were investigate for their *in vivo* activity against *Plasmodium cathemerium* and *Plasmidium lophurae* in ducklings and avian malaria, i.e. *Plasmodium gallinaceum* in chicks (Spencer *et al*., 1947; Adebayo and Krettli, 2011) [3]. This has resulted into waste of time, resources and posed a danger for the urgent need to find alternative and highly potent compounds for malaria treatment. Therefore, a healthier approach is the selection of plants based on their indigenous ethnobotanical information against malaria.

Although, several previous studies had reported a quite number of plants species used by local populace singly or in combination to prevent or treat malaria in Nigeria (Odugbemi *et al*. 2007; Ajibesin *et al*. 2008; Majekodunmi *et al*., 2008) [23, 8, 19], majority of this studies focused on southern part of the country where malaria transmission persist throughout the year and where the presence of tropical humid climate is believe to support growth of ethnobotanical. There remains paucity of investigation as regards to the medicinal plants used for malaria treatments in most northern state of Nigeria and no scientifically evidence so far to prove whether or not plants currently used folk medicine have antplasmodial properties *in vivo*, particularly in Kano which is pretty populated and despite the long history of malaria in northern Nigeria.

In continuation of search for antimalarial compounds/extract from medicinal plants, this study investigated antiplasmodial activities and phytochemical constituent of back extracts of three medicinal plant commonly used by indigenous people of Kano for malaria treatment. These included *Boswillia dalzielii* Hutch., *Dyospyros mespiliformis* (Hochst. ex A. DC), and *Ficus sycomorus* L. The three plant species are trees and have been documented to have ethnomedicinal properties (Table 1).

Table 1: List of plant species collected and some of their documented ethno-medicinal use

Botanical Name	Family	Parts collected	Collection Location	Documented Ethnomedical Use	Documented parts Used	References
Boswillia deilzeili Hutch.	Burseraceae	Bark	Gabasawa, Kano state	against dysentery, haemorrhage, angina, malaria, yellow fever, stomach ailmen, rheumatism, gastrointestinal disorders, wounds, asthma, pleurisy, appendicitis, dizziness, palpitations, leprosy, diarrhoea	Stem bark	Uzama *et al*. (2015) [35] Kohoude *et al*. (2017) [18], Mbiantcha *et al*. (2018) [20]
Dyospyros mespiliformis Hoschst ex A. D	Ebanaceae	Bark	Gabasawa, Kano state	treatment of fevers, pneumonia, syphilis, leprosy, headache, arthritis and skin infections, stomach ache, malaria, pneumonia, syphilis, leprosy, dermatomycoses,	Leaves, Root and Stem bark	Maydell, (1990), Ruffo *et al*. (2002), Mohamed *et al*. (2009) [21], Chivandi and Erlwanger, (2011)
Ficus sycomorus	Moraceae	Bark	Gabasawa, Kano state	Treatment of liver disease, pain killer, parasitic infections, coughs, a decoction of leaves is used in the treatment and management of high blood pressure	Stem back and leaves	Odugbemi and Akinwade, (2006), Shinkafi and Abdullahi, (2018) [29]

Boswillia dalzielii is a deciduous tree species of the family Burseraceae, growing up to 12m high. It is a common plant species north-western Nigeria. It is popular among the locals for it ethnomedicinal properties. Recent study has reported the stem bark of *B. dalzielii* to possess the property that significantly inhibit the production of pro-inflammatory cytokines (Mbiantcha *et al*. 2018) [20]. Decoction of the bark has been noted to be useful in treating dysentery, hemorrhage, and angina (Kohoude *et al*. 2017) [18]. Dried bark joined with other herbs are used for the treatment malaria, yellow fever, appendicitis, dizziness, stomach ailments, and gastrointestinal disorders (Uzama *et al*. 2015) [35]. Investigation of its phytochemical constituent revealed presence of tannins, flavonoids, cardiac glycosides, steroids, terpenes, among others (Adelakun *et al*., 2001) [4]. The methanol and aqueous extracts of *B. dalzielii* has broad-spectrum antibacterial and antifungal activities (Adelakun *et al*., 2001) [4].

Diospyros mespiliformis is a large deciduous tree with dense, rounded and buttressed stem. The Bark is grey-black or black, smooth in young trees and pinkish when slashed (Ahmed and Mahmud, 2017) [5]. It belongs to the Ebenaceae family and found mostly in tropical and sub-tropical regions of Africa (Mohamed *et al*. 2009) [21]. Phytochemical investigations of it extract revealed presence of substances similar to

scopolamine, tannins, saponins and several other secondary metabolites (Mohamed *et al*. 2009; Shagal *et al*. 2011; Abba *et al*. 2016) [21]. Ethanol extracts of stembark of *Diospyros mespiliformis is reported to possess* anti-inflammatory and antimicrobial activity (Dangoggo *et al*., 2016) [11].

Ficus sycomorus belongs to the family *Moraceae*, and it is widely spread in West Africa. It is a scrambling shrub with light bark, rough and hairy leaves, which are used in ancient Africa as sand paper for polishing woods (Shinkafi and Abdullahi, 2018) [29]. Decoction of the bark of *F. sycomorus* is useful for treating certain liver diseases and parasitic infection (Ahur *et al.*, 2010) [6]. In Africa traditional medicine, the leaves are useful for treating wounds, anthelmintic, coughs and purgative. The extract from *Ficus spp.* has been reported to possess several important phytochemical including Flavonoids, Tannins, Terpenoids, phytate, oxalate, among others (Bamikole *et al*., 2004; Usman *et al*., 2009) [9, 34].

This study compared *in vivo* antiplasmodial activity of the methanol extracts of the back of the three plants species and chloroquine phosphate on Adult Swiss albino mice, maintained under standard laboratory conditions. It is expected that the outcome of this study will guide intervention programme aimed at finding remedies for malaria eradication and add to the list of plants from which antimalaria drug can be formulated.

Materials and Methods

Collection of plant materials and Identification

Fresh stem back of *plant species; B. dalzielii, D. mespiliformis and F. sycomorus were collected* from Gabasawa Local Government Area in Kano state, following information gathered from the indigenous people as the most commonly used plant for malaria treatment. The plants were identified at Herbarium of the Department of Plant Biology, Bayero University Kano and voucher specimen was deposited. Voucher Number for species Identified are given as follows: BUKHAN 3362 (*B. dalzielii*), BUKHAN 121 (*D. mespiliformis*) and BUKHAN 109 (*F. sycomorus*).

Plant preparation and extraction

The back of the three tree species was air dried at room temperature for 7 days and pulverized to a coarse powder using an electric mill. The methanol extraction of the material was done by soaking 150 g of the powdered sample into 550 ml of methanol for 72 hours at 50°C in water bath. This process was done separately for each of the three species, and placed in separate labeled glass bottles. The mixtures were filtered and the filtrates were condensed under low pressure using a rotary evaporator at a temperature of 55°C. The pellet obtained was crushed into a powdery form and then subjected to crude methanol extract.

In vivo determination of antiplasmodial activity of the plant extract

Experimental animals

Fifty-five (55) pure strains of albino mice (Adult Swiss) with an average weight of 21g and of either sex were obtained from Nigeria Institute of Medical Research (NIMR), Yaba, Lagos. The mice were treated in compliance with the Guide for the Care and Use of Laboratory Animals as documented by the US National Institute of Health (NIH publication No. 85-23, revised 1985) and in line with the known ethical standards as approved by the University of Lagos Ethical Committee (IRB/19/002). The animals were allowed to acclimatized for 1 week before the commencement of treatment. They were monitored under 12 hours light/dark cycles, fed with mice pellets and allowed to have access to drinking water prior randomization into treatment groups.

Malaria Parasite and treatment suppressive activity after 4-days of infection

The NK65 strain of *Plasmodium berghei* which is sensitive to Chloroquine was used in this study. *P. berghei* was sourced from Dr Aina's Laboratory, Biochemistry and Nutrition Department, Nigerian Institute of Medical Research, Yaba. An infected donor mouse with the *P. berghei* strain was used for parasites inoculums preparation. Each mouse was injected with 0.1ml of the infected blood containing about 1×10^6 *P. berghei* parasitized red blood cells. The infected animals were randomized into 11 groups, with each group consist of 5 animals of similar weight. Antiplasmodial activities of the plant extracts and chloroquine were investigated using four (4) day suppressive test according to Peter and Anatoli (1998) [27]. The plant extracts and chloroquine were administered daily on the infected through the oral route using stainless metallic cannula. After 2 hours of parasite inoculation, the first nine groups were further divided into three sub-group and treated with the methanol extract of the three plant species at concentration 200mg/kg, 400mg/kg and 800mg/kg body weight. One of the remaining two groups serve as negative control was given only distilled. The last group was treated with Chloroquine and serve as positive control. The administration of extract and drug was repeated daily for 4 days. On day 4, a thin blood film was made from the tail of each mouse. The smear was prepared by spreading the blood on a clean slide over an area of 1.5 cm × 2.5 cm, allowed to dry and fixed with methanol. The slides were stained using 5% Giemsa for 30 minutes and examined with microscope. The percentage parasitaemia was obtained by enumerating parasitized red blood cells out of 500 erythrocytes in random fields of oil immersion objectives of Olympus CX microscope.

Percentage parasitaemia was calculated as;

$$\%\ Parasitaemia = \frac{NO.\ Of\ Parasitized\ RBC}{Total\ No.\ of\ RBC\ counted} \times 100$$

The average % suppression of parasitaemia in relation to the control was assessed using;

$$Av\ \%\ suppression = \frac{Av\ \%\ parasitaenia\ in\ control - Av\ \%\ parasitaenia\ in\ test}{Av\ \%\ parasitaenia\ in\ control} \times 100$$

Qualitative determination of the phytochemical's constituent

The pulverized back of the plants was extracted with solvent of increasing polarity (Diethyl Ether, Chloroform and Methanol) and screened for bioactive properties using the standard procedures as described by Sofowora (1993) [31]; Trease and Evans (2002) [33], and Harborne (1973) [14]. Briefly, 25g of powdered materials from each plant species was extracted successively with Diethyl Ether, Chloroform and Methanol for 18–20 h. The extracts were crystalized by rotary evaporator at 37 °C and preserved at 4 °C. All extracts were allowed to undergo qualitative chemical assessments in order to identify selected phytoconstituents. The qualitative results are expressed as (+) for the presence and (−) for the absence of phytochemicals. The percentage extraction yield was calculated as;

$$Extraction\ yield\ (\%) = \frac{weight\ of\ extract\ after\ evaporating\ solvent}{dry\ weight\ of\ the\ sample} \times 100$$

Test for tannins
The present of tannin in each extract was determined by ferric chloride test. Two milliliters (2 ml) of each back extract was stirred with some drops of ferric chloride (5%). Black or blue-green coloration or precipitate signify presence of tannins (positive result).

Test for Alkaloids
The present of alkaloids was determined using *Wagners test.* Two-gram (2 g) potassium iodide and iodine (1.27 g) were liquified in 5 mL distilled water. The solution was diluted to 100 mL with distilled water. Some drop of the solution was added to the back extract of the three plants; a precipitate with brown color signify presence of alkaloids.

Test for flavonoids
Magnesium chips (3 pieces) were added to 3ml of each filtrate, followed by some drop of concentrated HCl. A pink, orange, or red which will change to purple color indicate flavonoids.

Test for Glycosides
Glacial acetic acid (2 mL) with some drops of ferric chloride was added to 5 ml of the plant extract, followed by 1 ml H_2SO_4. The occurrence of brown ring at the interface signify cardiac glycoside and a violet ring may appear beneath the brown ring.

Test for steroids and Triterpenoids
The extracts were mixed with chloroform and little drops of acetic anhydride in a test tube. The samples were boiled in a water bath and swiftly cooled in iced water. Two milliliter (2ml) of concentrated H_2SO_4 was then added. The occurrence of brown ring at the intersection of two layers and changing of the upper layer to green imply existence of steroids while formation of deep red color implies presence of triterpenoids.

Test for Saponins and phenols
Extracts (5ml) of the three plants were independently shaken with10 mL distilled water. The occurrence of frothing, which continues on warming in a water bath for 5 min, demonstrate the presence of saponins. For the determination of presence of phenol, 500 mg of the extracts were dissolved in 5 ml of distilled water, followed by some drops of neutral 5 % ferric chloride solution. Observation of a dark green color implies that a phenolic compound is present.

Statistical analysis
Data obtained for % parasitaemia in *P. berghei* NK65-infected mice were subjected to One-way analysis of variance (One-way Anova). Analyses were performed in SPSS version 18.0 (SPSS, Chicago, Illinois, USA). Data are presented as Mean ± standard deviation. Differences at $p < 0.05$ were considered significant. Figures were prepared using SigmaPlot 12.5 (Systat Software, Erkrath, Germany).

Results
Antiplasmodial Activities of the back extract of the three plant species
The *in vitro* investigation of the methanol back extract on established P. berghei NK65-infected mice showed that the antimalaria activities of the three plant species are dose dependent (Table 2). At the dose of 800 mg/kg body weight, *F. sycomorus* and *D. mespiliformis* had higher antimalaria activities as indicated by the lower average % parasitaemia and higher % suppression when compared with the *B. dalzielii* and negative control (Table 2 and 3). The back extract of *D. mespiliformis* at 400 mg/kg body weight and *F. sycomorus* at 200 mg/kg body had average % parasitaemia of 4.92% and 4.38%, and correspond to 36.36 % and 22.81 % average % suppression, respectively. Chloroquine (CQ) as the positive control had 88.89 % suppressive effect. The overall comparism of % parasitaemia among the three plants showed that while there was no significant different among *B. dalzielii, D. mespiliformis* and *F. sycomorus* at 200 and 400 mg/kg body, *B. dalzielii* had significantly ($P < 0.05$) high % parasitaemia (Fig 1), indicating very low antimalaria activities compared to the other two plants.

Table 2: Average % parasiteamia in *P. berghei* NK65-infected mice treated with crude methanol back extract of *B. deilzeili, D. mesopiliformis* and *F. sycomorus*

Plant species	Dose (mg/kg body weigh)		
	Methanol		
	200	400	800
B. deilzeili	6.39± 2.12	7.76±2.15	7.26±2.67
D. mesopiliformis	6.48± 1.05	4.92± 1.09	3.6± 1.05
F. sycomorus	4.38± 0.76	5.32±0.79	1.95±0.71
	Negative control	Positive control, (CQ)	
Control Treatments	8.24±2.29	0.82±0.04	

Table 3: Average percentage suppression of parasite by the crude methanol back extract of *B. deilzeili, D. mesopiliformis* and *F. sycomorus*

Plant species	Dose (mg/kg body weigh)		
	Methanol		
	200	400	800
B. deilzeili	21.95	4.89	10.98
D. mesopiliformis	15.58	36.36	53.25
F. sycomorus	22.81	7.02	65.79
	Negative control	Positive control, (CQ)	
Control Treatments	0.00	88.89	

Percentage yield of the back extract in solvent of increasing polarity and the corresponding phytochemical constituent
To investigate the impact of solvent used for extraction on the phytochemical constituent, we selected three most common solvent of different polarity, which are diethyl ether, chloroform and methanol. Each solvent yield different quantity of the crude extract from the back of the three plant (Table 4). Among the three solvent, extraction methanol generated highest percentage yield which was found to be 19 % in *B. dalzielii*, 10 % in *D. mespiliformis* and 4 % in *F. sycomorus*. The use of Chloroform resulted into 2 % yield from *B. Dalzielii*, 1.3 % from *D. mespiliformis* and 1.03 % from *F. sycomorus*. Using diethyl ether, the percentage yield from all the plant extract were found to be 1.02, 3.82 and 1.27 % from *B. Dalzielii, D. mespiliformis* and *F. sycomorus* respectively (Table 4). All extracts were investigated for the existence of phytochemical constituent.

Table 4: Percentage yield of the plants extract and their colour in different solvents

Plants species	Fractions					
	Dee		CF		MeOH	
	Yield	Colour	Yield	Colour	Yield	Colour
B. deilzeili	1.02	green	2	Dirty green	19	Dark red
D. mesopiliformis	3.82	brownish	1.3	Light brown	10	red
F. sycomorus	1.27	brown	1.03	green	4	red

DEE: Diethyl Ether, CF: Chloroform and MeOH: Methanol

The methanolic extract of the back of the three species showed the present of flavonoid and phenol (Table 5). Alkaloids and triterpenoids were detected in methanol extract of *D. mespiliformis*, but only triterpenoid was present in *B. Dalzielii*. For *F. sycomorus*, Alkaloids and Glycosides were detected in chloroform extract but absent in diethyl ether extract. On the other hand, steroids and triterpenoids were present in diethyl ether extract and absent in chloroform and methanol extract. Flavonoids and saponin were present in the chloroform extract of *D. mespiliformis*, but absent in diethyl ether extract. Except for flavonoids and phenol, all the secondary metabolites tested were present in chloroform extract of *B. Dalzielii* (Table 5). In diethyl ether extract of *B. Dalzielii*, flavonoids, saponins and phenols were absent.

Table 5: Phytochemical constituents of crude extract of the back of the three plant species using solvent of increasing polarity

	B. deilzeili			***D. mesopiliformis***			***F. sycomorus***		
Secondary metabolites	**DeE**	**CF**	**MeOH**	**DeE**	**CF**	**MeOH**	**DeE**	**CF**	**MeOH**
Alkaloids	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve
flavonoids	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve
Glycosides	+ve	+ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve
Steroids	+ve	+ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve
Triterpenoids	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve
Saponins	-ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve
phenols	-ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve
Total Secondary metabolites	4	5	3	3	3	4	3	3	2

DEE: Diethyl Ether, CF: Chloroform and MeOH: Methanol

Discussion

Although recent studies have documented potency of some indigenous plant species for malaria treatment (Adebayo and Krettli, 2011; Kigondu *et al*. 2011; Bankole *et al*. 2016) [2, 17, 10], the present study focused on, and compared *in vivo* antimalaria activities and phytochemical constituent of *Boswillia dalzielii*, D. *mespiliformis* and *F. sycomorus* which are indigenous plants mostly used in northern Nigerian folk medicine. The *in vivo* investigation of the antimalaria properties of the methanol back extract of three species indicated their potency in combatting malaria infections. The extract from the three plant species showed significant chemo suppression against *P. berghei* in tested mice compared to the mice in negative control. However, the level of potency differed among the plant species and was also dose dependent. Specifically, the present finding indicated that among the three species, methanol back extract of *F. sycomorus* and *D. mespiliformis* at higher dose of 800 mg/kg body weight possess quality malaria suppression properties, whereas extract from *B. dalzielii* has very minimal suppression effects on the parasite growth. The observed little effect of *B. dalzielii* extract on malaria parasite at higher dose is contrary to the assertion of Salihu *et al*. (2020) [28] that this plant possesses very good and potent antimalaria properties. Instead, our finding revealed that the potency of *B. dalzielii* extract on the growth of malaria parasite is good only at lower dose of 200 mg/kg body weight, thus suggesting dose selectivity effects and the need for investigation into its cytotoxicity before using for malaria treatment.

The remarkable effects of *F. sycomorus* and *D. mespiliformis* back extract at higher does is consistent with several previous study on other plant species (Ajala *et al*. 2011; Bankole *et al*. 2016) [7, 10]. For instance, Bankole *et al*. (2016) [10] when investigating antimalaria activities of *M. tomentosa* reported that at dose of 800 mg/kg body weight, *P. berghei* was significantly suppressed in the tested mice but this was not the same at lower dose. In addition to several previous studies who have documented medicinal use of leave, stem back and root extract of *Boswillia dalzielii*, D. *mespiliformis* and *F. sycomorus* in treating various diseases including dysentery, hemorrhage, fevers, pneumonia, liver disease, coughs and several others (Mohamed *et al*. 2009; Kohoude *et al*. 2017; Mbiantcha *et al*. 2018; Shinkafi and Abdullahi, 2018) [21, 18, 20, 29]. This study revealed the potency of the methanol extract of the three plant species in malaria treatment and thus may be a great source of new antimalaria drugs to combat the continuous prevalence of malaria infections.

Given the beneficial importance of plant-derived antioxidants on human health and the subsequent role of extracting agent in determine their presence or absent (Okunlola *et al*. 2017; Wakeel *et al*. 2019) [24, 25], we compared the effect of diethyl ether, chloroform and methanol on the yield and presence of some selected phytochemical in the three plant species. The present findings indicated that the extraction effectiveness differed among the solvent and favors the highly polar solvents. Compared with diethyl ether and chloroform, extraction with methanol produced highest yield, suggesting that plant material from the three plant species contains high levels of polar compounds that are soluble in solvents with high polarity. Furthermore, the qualitative investigation of the three plant species showed that the present or absence of secondary metabolites varies among the plants and depends on the type of solvent used for extraction. Alkaloids, flavonoids, glycosides, triterpenoids and phenols were present in all the three species which was in consistent with previous studies (Younoussa *et al*. 2016; Mbiantcha *et al*. 2018) [40, 20], but sometimes in different extracting solvent. For instance, we found that while flavonoid was present only in the methanol extract of *B. dalzielii*, it was present in chloroform and methanol extract of *D. mespiliformis* and present in *F. sycomorus* irrespective of the extracting solvent. The occurrence of flavonoids in *D. mespiliformis* and *F. sycomorus* in majority of the extract solvent give credence to their earlier observed better effectiveness in combating malaria parasite as compared to *B. dalzielii*. Although, flavonoids are mostly considered as non-nutrients, they are essential to the human health due to their effectiveness in scavenging reactive oxygen species which comprised of singlet oxygen and other free radicals (Okunlola *et al*. 2019) [25].

Contrary to a previous study that reported presence of saponin and other phytochemicals in *F. sycomorus* (Hassan *et al*. 2006) [15], the results from the presence study showed that while other phytochemicals reported by Hassan *et al*. (2006) [15] are also present, saponin is completely absent irrespective of the solvent used. Similarly, Steroids are completely absent in *D. mespiliformis*, but present in *B. dalzielii* and *F. sycomorus* at varying solvent. Steroids are known to be

abundant in many plants and function in mediating cardiotonic activities, possess insecticidal and antimicrobial properties (Okunlola *et al*. 2017) [24], while saponin typically used in treatment of hypercholesterolemia, hyperglycemia, and anticancer (Okunlola *et al*. 2019) [25]. Nevertheless, the present of all aforementioned antioxidant in the back extract of all the three species despite in different extracting solvent may be responsible for the biological activities against malaria infection and the reason for their use in traditional medicine by the indigenous people of Kano as well as in most northern state of Nigeria.

Conclusion

The findings from this study established that though the methanol back extracts of the three investigated plant are potent against malaria infection, *D. mespiliformis* and *F. sycomorus* are more potent compared with *B. dalzielii* and can be considered when developing antimalarial drug. The result obtained further indicated that the extract from the plant are more potent at higher dose. Although the present of secondary metabolites depends on the type of solvent used for extraction, some phytochemical including Alkaloids, flavonoids, glycosides, triterpenoids and phenolic compounds were present in all the plant species investigated. Further investigation is ongoing to isolate, assay and elucidate on structural similarity or differences of the key compound in the three plant species.

Disclosure statement

No potential conflict of interest was reported by the authors.

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