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Antiplasmodial activities of n-hexane and water fractions of *Dennettia tripetala* leaf extract in Wistar rats

Mba Ogbonnaya [1], Chibueze P. Ihekwereme [1,*], Uchechukwu H. Orji [1] and Aziakpono M. Omoirri [2]

[1] *Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria.*
[2] *Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Federal University of Ayo-Ekiti, Ekiti State, Nigeria.*

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Abstract

Many people die from malaria, a global health crisis. Most global malaria cases and deaths occur in sub-Saharan Africa. The rising resistance of *Plasmodium spp*. to antimalarial medications is hindering efforts to eradicate the illness. This study tested *Dennettia tripetala* leaf extract, N-hexane, and water fractions for antimalarial activity in rats. We used standard methods to screen *Dennettia tripetala* solvent (N-hexane and water) for phytochemical components. We tested the antimalarial potential of rats using Peters' 4-day suppressive, Rane's curative, and prophylactic tests. After conducting an exhaustive investigation, it was discovered that both fractions contained varying amounts of flavonoids, alkaloids, terpenoids, and saponins. There was a significant amount of antimalarial activity demonstrated by both fractions across all test models ($P<0.05$). Compared to the water fraction, *Dennettia tripetala*'s n-hexane fraction was highly chemosuppressive and curative. *Dennettia tripetala* n-hexane has chemoprophylactic, curative, and chemosuppressive effects. The curative effect was 62.3% to 72.4%, and the chemosuppressive effect was 51.2% to 88.5%. Chemoprophylactic activity was 32.1%–61.2%. At 250–1500 mg/kg, *Combretum nigricans* butanol suppressed 40.3%, 54.1%, and 69.1%, respectively. Therapeutic effects were 26.2%, 36.9%, and 34.5%, while chemoprophylaxis was 48.4%, 70.0%, and 87.4%. Both *Dennettia tripetala* solvent fractions have antimalarial activity, suggesting they may be effective in different malaria therapy stages.

Keywords: Antimalarial; Chemoprophylaxis; Chemosuppressive; *Dennettia tripetala*

1. Introduction

Malaria, a severe feverish disease, continues to be one of the most significant worldwide health issues, with around 250 million cases and over 600,000 deaths each year [1]. According to a recent assessment by the WHO, the sub-Saharan African region is responsible for an exceptionally high percentage (about 90%) of global malaria infections and deaths (around 91%) [2]. Many resources and efforts have been made to get rid of the disease, but the parasite Plasmodium sp. is becoming more and more resistant to the antimalarial drugs that are currently available [3]. The global prevalence of antimalarial drug resistance in *Plasmodium* parasites, particularly *Plasmodium vivax* and *Plasmodium falciparum*, has increased over the last 50 years [4]. Nevertheless, despite the significant rise in resistance to existing drugs, there has been a lack of new and effective drug launches. Over ten years have passed since the introduction of a new antimalarial drug to the market [5]. Therefore, without immediate action to introduce new and effective antimalarial drugs, there is a potential danger of *Plasmodium* strains resistant to current treatments spreading worldwide. Several medicinal plants, in addition to traditional medicines, treat a wide range of disorders in Africa. According to statistics, almost 80% of the African population uses herbal medicine to treat various illnesses, such as malaria [6]. It is critical to scientifically

* Corresponding author: Chibueze P. Ihekwereme

evaluate the stated effectiveness of these plants, despite reports suggesting their potency. Furthermore, conducting pharmacological assessments of medicinal plants has the potential to unveil innovative drugs. Empirical evidence from medical history has conclusively demonstrated that plants play a crucial role in the identification and development of novel pharmaceuticals [7]. Nigerian ethnomedicine uses *Dennettia tripetala*, among other medicinal herbs, as a malaria remedy. The goal of this study is to evaluate the efficacy of *Dennettia tripetala's* n-hexane and water fractions in treating malaria in rats.

2. Materials and methods

2.1. Plant materials

Dennettia tripetala leaves were collected from Ekwulobia, Anambra state, Nigeria, and were identified and authenticated by Dr. Onoriode Peterson, a taxonomist, at the Department of Botany at Delta State University Abraka, Nigeria.

2.2. Extraction

We desiccated the plant leaves at ambient temperature for two weeks and then ground them into a fine powder. We soaked the crushed leaves in ethanol, following the established methods outlined by Omoirri et al. [8We filtered the resulting combination 72 hours later using a muslin cloth, followed by Whatman filter paper (No. 1), and subsequently concentrated it. We subjected the leaf extract to bioactivity-guided fractionation using n-hexane and water as solvents. This process involved solvent-solvent partitioning, following the established protocols described by Omoirri et al. [9] and Omoirri et al. [10]. We stored the collected n-hexane and water fractions in a refrigerator at 4 degrees Celsius until they were ready for use.

2.3. Phytochemical analysis

N-hexane and water fractions of *Dennettia tripetala* were screened for their phytochemical constituents using standard procedures illustrated by Harborne [11] and Evans [12].

2.4. Animals

The study utilized both male and female albino rats, which had an average weight of 30.2±1.22 grams. The Department of Pharmacology and Toxicology at Nnamdi Azikiwe University maintained these rats at their animal facility. The Department of Pharmacology and Toxicology at Nnamdi Azikiwe University confined the rats in enclosures, maintained in typical laboratory settings at ambient temperature and humidity, with unrestricted availability of water and rat food. We conducted the animal research in compliance with the "NIH revised guidelines for laboratory animal care and use" [13] and the ethical codes and regulations for laboratory animal use at Nnamdi Azikiwe University in Awka.

2.5. Rodent parasite

We acquired the *Plasmodium berghei* (P. berghei) parasite, which causes malaria in rodents, from the Faculty of Veterinary Medicine at the University of Nigeria, Nssuka. We kept the parasite alive in rats by repeatedly injecting blood from one rat into the peritoneal cavity of another rat.

2.6. Parasite inoculation

We produced a standard inoculum from the parasitized rat, which contained approximately 1x107 *P. berghei*-infected erythrocytes per milliliter. Each rat in the experiment received an intra-peritoneal injection of 0.2 mL of the inoculum.

2.7. Experimental design/ grouping and dosing of animals

The experimental animals were divided into eight groups, comprising five animals each. The treatment schedule was as follows:

- Group 1: Control (received distilled water 10 mL/kg b.w. p.o.)
- Group 2: treated with *Dennettia tripetala* n-hexane fraction 250 mg/kg b.w. p.o.
- Group 3: treated with *Dennettia tripetala* n-hexane fraction 500 mg/kg b.w. p.o.
- Group 4: treated with *Dennettia tripetala* n-hexane fraction 1500 mg/kg b.w. p.o.
- Group 5: treated with *Dennettia tripetala* water fraction 250 mg/kg b.w. p.o.
- Group 6: treated with *Dennettia tripetala* water fraction 500 mg/kg b.w. p.o.

- Group 7: treated with *Dennettia tripetala* water fraction 1500 mg/kg b.w. p.o.
- Group 8: treated with the standard antimalarial drug, Chloroquine 15 mg/kg b.w. p.o.

The same grouping and treatment were employed in the three test models used in the study.

2.8. Antimalarial studies

2.8.1. Activity on early malaria infection (suppressive test)

We assessed the efficacy of the first infection against *P. berghei* in mice using Peter's 4-day suppressive test [14]. Day 0 of the investigation involved injecting 0.2 mL of a standard inoculum of parasite-infected erythrocytes intraperitoneally into the animals, as previously described. We categorised the mice into groups, as shown above, and subjected them to treatment for a continuous period of 4 days (D0–D3). We started the treatment within 3 hours after inoculating the mice with the parasite. On the fourth day of the investigation (D4), we created a thin film using blood samples from the tails of each experimental mouse. We placed the film on a microscope slide. We scrutinised the slides under a microscope, adhering to Cheesbrough's [15] method, to determine the average parasitemia. We quantified the chemosuppression of parasitemia as a percentage using the formula (A-B/A) x 100, where A represents the average parasitemia in the negative control group and B represents the average parasitemia in the treatment group.

2.8.2. Activity on established infection (Rane's test)

We assessed the therapeutic efficacy of *Dennettia tripetala* n-hexane and water fractions against an existing infection using the Ryley and Peters [16] described methodology. The trial began with the division of the animals into eight groups. As previously explained, we injected them into the peritoneal cavity and did not administer any treatment until the fourth day of the study (D3). We created a thin film on the third day (D3) using blood samples from each experimental mouse's tail to determine the number of parasites present before any therapy. The mice had a four-day treatment period from day 3 to day 6. We created a thin film on the eighth day (D7) using blood samples from each experimental mouse's tail to measure the level of parasites after treatment. We determined the percentage of erythrocyte parasite clearance using the formula (X – Y/X) x 100, where X represents the average parasitemia on day 3 before treatment and Y represents the average parasitemia on day 7 after treatment. We subjected the mice to additional monitoring to determine the average duration of survival (in days) for each group of animals. We monitored each group of animals daily for mortality starting from day 0. This monitoring continued after the treatment period until all of the animals died.

2.8.3. Evaluation of prophylactic activity (repository test)

Peters [17] described a residual infection approach to evaluate the preventive effectiveness of *Dennettia tripetala* n-hexane and water fractions. We categorized the animals as previously depicted on the initial day of the investigation (D0). The animals had daily pre-treatment for four consecutive days (D0–D3) prior to parasite inoculation. The mice received a standard inoculum containing *P. berghei*-infected erythrocytes on the fifth day of the trial (D4). We took a blood sample from each mouse's tail at the 72-hour mark following treatment (on day 6) and analyzed it under a microscope to measure the level of parasitemia. We determined the chemoprophylactic impact, expressed as the percentage of chemoprophylaxis, using the formula (A minus B/A) x 100. Here, A represents the average parasitemia in the negative control group, and B represents the average parasitemia in the treatment group. We measured the animals' body weight in the treated groups and control group on the first day of the research (D0) before treatment began, and again on D6, using a precise digital weighing balance. We determined the change in body weight (g) by subtracting the mean body weight on day 0 from the mean body weight on day 6.

2.9. Statistical analysis

The study presented the data as the mean value plus or minus the standard error of the mean (SEM). The statistical methods employed in this study were one-way analysis of variance (ANOVA) and Dunnett's post hoc test. A significance level of $P<0.05$ was used. The study was conducted using GraphPad Prism for Windows (version 7.0), developed in San Diego, California, USA.

3. Results

3.1. Phytochemical screening

The phytochemical screening revealed the presence of alkaloids, terpenoids, saponins, flavonoids, and other phytochemicals in *Dennettia tripetala* n-hexane and water fractions. The phytochemicals were Present at different intensities in both fractions (Table 1)

Table 1 The phytochemical constituents present in *D. tripatala*

Phytoconstituents	N-hexane fraction	Water fraction
Saponins	++	+
Steroids	++	++
Terpenoids	+++	++
Resins	+	-
Tannins	++	+++
Flavonoids	+++	++
Cardiac glycosides	+++	+
Carbohydrates	+	+
Proteins	++	++
Quinones	++	+++
Reducing sugar	++	++

+++ = abundantly present; ++ = moderately present; + = present; - = absent.

3.1.1. Activity on early malaria infection (suppressive test)

The *Dennettia tripetala* n-hexane and water fractions at all test doses elicited significant (P<0.05) chemosuppressive effect (Table 2).

Table 2 Chemosuppressive activity of *Dennettia tripetala* n-hexane and water fractions

Treatment	Dose (mg/kg)	Mean parasite count	Chemosuppression (%)
Control (water)	10 mL/kg	18.75 ± 2.17	0
DTHF	250	5.10 ± 0.58*	51.2
	500	4.14 ± 0.17*	76.1
	1000	2.30 ± 0.11*	88.5
DTWF	250	11.20 ± 1.39*	40.3
	500	8.60 ± 1.21*	53.5
	1000	5.80 ± 0.80*	79.3
Chloroquine	15	5.80 ± 1.32*	85.1

Values expressed as Mean ± SEM, n=5, *compared with the control group, the difference is significant at P<0.05, DTHF = *Dennettia tripetala* n-hexane fraction, DTWF = *Dennettia tripetala* water fraction.

3.1.2. Activity on established infection (Rane test)

Both solvent fractions of *Dennettia tripetala* demonstrated significant (P<0.05) antimalarial activity (Table 3). *Dennettia tripetala* n-hexane fraction at doses of 500 and 1000 mg/kg and water fraction at all doses prolonged the mean survival time of the animals (Table 3).

Table 3 Curative effect of *Dennettia tripetala* n-hexane and water fractions

Treatment	Dose (mg/kg)	Mean parasitemia		Erythrocyte parasite clearance (%)	Mean survival time (Days)
		D3	D7		
Control (water)	10 mL/kg	27.13 ± 0.45	35.80 ± 1.43	-33.8	10.8
DTHF	250	27.18 ± 2.89	10.10 ± 1.26*	62.3	11.0
	500	26.22 ± 0.86	8.11 ± 1.50*	75.3	15.5
	1000	24.50 ± 1.63	5.13 ± 0.91*	87.4	18.5
DTWF	250	26.53 ± 1.68	19.00 ± 0.65*	26.1	8.5
	500	25.12 ± 1.21	15.67 ± 3.28*	55.9	13.3
	1000	24.10 ± 0.02	14.50 ± 0.50*	77.5	18.1
Chloroquine	15	24.10 ± 0.92	5.04 ± 0.65*	88.7	22.5

Values expressed as Mean ± SEM, n=5, *compared with control group, difference is significant at P<0.05, DTHF = *Dennettia tripetala* n-hexane fraction, DTWF = *Dennettia tripetala* water fraction.

3.1.3. Prophylactic activity (repository test)

Both *Dennettia tripetala* fractions had significant (P<0.05) chemoprophylactic effects in a dose-related trend. The fractions prevented decrease in body weight at doses of 25, 500 and 1000 mg/kg. Artesunate also had a preventive effect against loss in mice body weight, while the control group mice had a remarkable decrease in body weight (Table 4).

Table 4 Chemoprophylaxis effect of *Dennettia tripetala* n-hexane and water fractions

Treatment	Dose (mg/kg)	Mean parasite count	Chemo-prophylaxis (%)	Weight (g)		Weight change (g)
				D_0	D_6	
Control (water)	10 mL/kg	27.70 ± 1.45	0	19.82 ± 0.42	16.60 ± 1.00	-3.22
DTHF	250	15.30±1.20*	44.77	19.11 ± 0.59	19.55 ± 0.33	0.44
	500	10.16 ±0.35*	63.32	19.14 ± 0.68	20.67 ± 0.28	1.53
	1000	4.46 ± 0.22*	84.00	19.10 ± 0.87	21.10 ± 1.35	2.00
DTWF	250	19.12 ±1.36*	30.97	20.08 ± 0.41	20.31 ± 0.75	0.23
	500	13.25 ±0.75*	52.17	21.11 ± 0.95	22.23 ± 2.21	1.12
	1000	5.09 ± 1.38*	81.62	18.81 ± 0.57	20.06 ± 0.28	1.79
Chloroquine	15	4.17 ± 0.87*	84.95	19.95 ± 1.34	22.55 ± 1.66	2.60

Values expressed as Mean ± SEM, n=5, *compared with the control group, the difference is significant at P<0.05, DTHF = *Dennettia tripetala* n-hexane fraction, DTWF = *Dennettia tripetala* water fraction.

4. Discussion

The phytochemical investigation revealed the presence of numerous secondary metabolites, known for their antimalarial properties, in both the butanol and ethylacetate fractions of *C. nigricans*. The study on antimalarials found that the butanol part of *C. nigricans* had a selective effect on the rat malaria parasite *P. berghei*, which changed depending on the dose. The ethylacetate part of *C. nigricans* had a stronger effect on chemosuppression. At a dose of 800 mg/kg, it had the highest level of activity (86.1%). This was better than the best chemosuppressive activity seen with the butanol fraction (69.1%) and artesunate, which was used as a standard. The ethylacetate fraction of *C. nigricans* has shown a

superior curative effect compared to both the butanol fraction and artesunate. During the trial, a dose of 800 mg/kg achieved the maximum erythrocyte parasite clearance of 72.4%. Nevertheless, the control group experienced a 33.8% rise in erythrocyte parasite levels. This suggests that mice, like humans, will experience *Plasmodium* parasite proliferation unless a potent antimalarial drug hinders it. This sequence of events will result in the appearance of malaria clinical symptoms and, ultimately, the animal's death for a shorter duration (i.e., reduced survival time). We anticipate that powerful antimalarial agents at therapeutic doses will extend the survival time of *P. berghei*-infected mice. Therefore, we regard as potent antimalarial agents any potential agents that can extend the survival time of *P. berghei*-infected mice beyond 12 days from the day of infection [5, 18]. The average length of time that animals in each group lived was much longer after they were given the butanol fraction of *C. nigricans* at doses of 400 and 800 mg/kg and the ethylacetate fraction of *C. nigricans* at all doses. This suggests that the solvent fractions of *C. nigricans* exhibit therapeutic efficacy. Artesunate demonstrated a longer average survival time, confirming its effectiveness as a commonly used conventional treatment. The butanol fraction of *C. nigricans* demonstrated superior chemoprophylactic activity in comparison to the ethylacetate fraction. In terms of effectiveness, the chemoprophylactic activity at 800 mg/kg was better (87.4%) than the activity seen with artesunate (83.4%). The butanol fraction's limited therapeutic efficacy may be due to its delayed onset of action compared to the ethyl acetate fraction. The butanol component of C. nigricans may have enhanced the animals' immune system to eliminate the malaria parasite, indicating its potential immunomodulatory impact. The parasite may have overwhelmed the immune system before treatment initiation, resulting in lower efficacy compared to its chemosuppressive and preventive actions, which may account for the reduced curative activity in this case. The butanol fraction of *C. nigricans* exhibits remarkable chemoprophylactic activity due to its low metabolism rate, hepatic clearance, and longer plasma half-life, among other pharmacokinetic features [19]. However, we have not fully understood the specific bioactive compound(s) responsible for the antimalarial effects of the solvent fractions, nor the underlying mechanism of action. It is known, however, that the presence of saponins, alkaloids, flavonoids, and terpenoids in the plant extract may play a role in this activity. Previous reports have indicated that they have antiplasmodial action [20–22]. Furthermore, researchers found a significant reduction in the body weight of the control rats. The *Plasmodium* infection, a recognised clinical sign of the illness, may have caused the drop in body weight. Nevertheless, this measure does not exclusively indicate a specific characteristic of malaria infection, as other reasons can also cause a reduction in body weight. While the 200 and 400 mg/kg dosages of *C. nigricans* solvent fractions prevented the decline in body weight, the 800 mg/kg dose of both fractions resulted in a modest drop in body weight. However, this decrease was significantly less than the loss observed in the control group. The solvent fractions of *C. nigricans* may have attributed the observed decrease in body weight to their catabolic activity on stored lipids or their anorexogenic effect, which could have led to reduced food consumption. Therefore, these fractions, in addition to their antimalarial properties, may potentially serve as anti-obesogenic drugs when administered at high doses of up to 800 mg/kg. Recent results from other researchers align with this finding, suggesting that certain plant substances, also known for their antimalarial effects, possess anti-obesogenic properties. According to their reports, they linked this activity to the existence of flavonoids, saponins, and other secondary metabolites in these plant agents [23–25].

5. Conclusion

This study has shown that the butanol fraction of *C. nigricans* has better chemoprophylactic activity against malaria infection, while the ethylacetate fraction has better chemosuppressive and curative antimalarial activities. Hence, both fractions may be useful at different stages of malaria therapy.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest is to be disclosed.

Statement of ethical approval

Ethical approval: Antiplasmodial activity of n-hexane and water fractions of *Dennettia tripetala* leaf extract in rats was approved by members of Nnamdi Azikiwe University Animal Research Ethics Committee (NAU-AREC) with approval number, NAU/AREC/2023/00078.

References

[1] Aliyu S. Viral, fungal, protozoal and helminthic infections. In: Laurence DR, Bennett PN, Sharma P (eds.) Clinical pharmacology. 11th ed. New York: Churchill Livingstone; 2012, p. 232.

[2] World Health Organization. Malaria. Geneva, Switzerland; 2016. [Online]. Available from: http://www.who.int/mediacentre/factsheets/fs094/en/. [Accessed on 2nd March 2024].

[3] Bamunuarachchi GS, Ratnasooriya WD, Premakumara S, Udagama PV. Artemisia vulgaris L. ethanolic leaf extract reverses thrombocytopenia/thrombocytosis and averts end-stage disease of experimental severe Plasmodium berghei murine malaria. J Vector Borne Dis 2014; 51(4): 286-293.

[4] Vinetz MJ. Chemotherapy of malaria. In: Brunton L, Hilal-Dandan R, Knollmann B (eds.) Goodman and Gilman's the pharmacological basis of therapeutics. 13th ed. New York, USA: McGraw-Hill; 2018, p. 969-985.

[5] Mulisa E, Girma B, Tesema S, Yohannes M, Zemene E, Amelo W.Evaluation of in vivo antimalarial activities of leaves of Moringa oleifera against Plasmodium berghei in Mice. Jundishapur J Nat Pharm Prod 2018; 13(1): e60426.

[6] Chinedu E, Arome D, Ameh SF. African herbal plants used as anti\malarial agents - A review. Pharma Tutor 2014; 2(3): 47-53.

[7] Chinedu E, David A, Ameh S. Phytochemical evaluation of the ethanolic extracts of some Nigerian herbal plants. Drug Dev Ther 2015; 6(1): 11.

[8] Omoirri M. A., Iloh S. E., Madubogwu N. U., Ajegi I. F and Eje V. I. Free radical scavenging activities of anthocyanin flavonoid. World Journal of Biology Pharmacy and Health Sciences 2020; 04 (03), 013–020

[9] Kupchan SM, Britton RW, Ziegler MF, Sigel CW. Bruceantin, a new potent antileukemic Simaroubolide from Brucea antidysenterica. J Org Chem 1973; 38(1): 178-179.

[10] Pollack A, Mansoor A. Hypofractio nation: Scientific concepts and clinical experiences, volume 1. 1st ed. Edinburgh: Churchill Livingstone; 2011.

[11] Harborne JB. Phytochemical methods. A guide to modern techniques of plant analysis. 2nd ed. London: Chapmann and Hall Publishers; 1998.

[12] Evans WC. Trease and Evans pharmacognosy. 14th ed. London: WB Saunders Company Limited; 2005.

[13] National Institute of Health. Guide for the care and use of laboratory animal (Revised). Washington: NIH Publication; 1985, p. 83-123.

[14] Peters W. The chemotherapy of rodent malaria, XXII. The value of drug-resistant strains of P. berghei in screening for blood schizontocidal activity. Ann Trop Med Parasitol 1975; 69(2): 155-171.

[15] Cheesbrough M. District laboratory practice in tropical countries. 2nd ed. UK: Cambridge University Press; 2004, p. 239-258.

[16] Ryley JF, Peters W. The antimalarial activity of some quinolone esters. Ann Trop Med Parasitol 1970; 64(2): 209-222.

[17] Peters W. Drug resistance in Plasmodium berghei vincke and lips, 1948. triazine resistance. Exp Parasitol 1965; 17(1): 90-96.

[18] Ural IO, Kayalar H, Durmuskahya C, Cavus I, Ozbilgin A. In vivo antimalarial activity of methanol and water extracts of Eryngium thorifolium Boiss (Apiaceae Family) against P. berghei in infected mice. Trop J Pharm Res 2014; 13(8): 1313-1317.

[19] Salawu OA, Tijani AY, Babayi H, Nwaeze AC, Anagbogu RA, Agbakwuru VA. Anti-malarial activity of ethanolic stem bark extract of Faidherbia albida (Del) a. Chev (Mimosoidae) in mice. Arch Appli Sci Res 2010; 2(5): 261-268.

[20] Alli LA, Adesokan AA, Salawu OA, Akanji MA, Tijani AY. Anti plasmodial activity of aqueous root extract of Acacia nilotica. Afr J Biochem Res 2011; 5(7): 214-219.

[21] Chierrito TP, Aguiar AC, de Andrade IM, Ceravolo IP, Gonçalves RA, de Oliveira AJ, et al. Anti-malarial activity of indole alkaloids isolated from Aspidosperma olivaceum. Malar J 2014; 13(1): 142.

[22] Christensen SB, Kharazmi A. Antimalarial natural products: Isolation, characterization and biological properties. In: Tringali C (ed.) Bioactive compounds from natural sources: Isolation, characterization and biological properties. London: Taylor and Francis; 2001, p. 379-432.

[23] Gooda Sahib N, Saari N, Ismail A, Khatib A, Mahomoodally F, Abdul Hamid A. Plants' metabolites as potential antiobesity agents. Sci World J 2012; 2012: 436039.

[24] Williams DJ, Edwards D, Hamernig I, Jian L, James AP, Johnson SK, et al. Vegetables containing phytochemicals with potential anti-obesity properties: A review. Food Res Int 2013; 52(1): 323-333.

[25] Mukherjee A, Mukherjee S, Biswas J, Roy M. Phytochemicals in obesity control. Int J Curr Microbiol App Sci 2015; 4(4): 558-567