



Effect of *Chrysophyllum albidum* Ethanol Leaf Extract on Haematological and Platelet Indices of Apparently Healthy Wistar Rat

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ABSTRACT

Chrysophyllum albidum ethanol leaf extract was studied to ascertain its potentials on hematological indices and hepatocyte of wistar albino rat. Twenty-five rats that weighed 98-168g were separated into five groups. Phytochemicals were quantified using standard methods. Alkaloids, flavonoids, tannins, saponins and terpenoids were detected. The leaf extract had 0.415 % of alkaloids, 0.102 mg/TAEq of tannins, 0.211 mg/QEq of flavonoids and 0.360 mg/GAEq of phenolics. Phytol, oleic acids, hexadecanoic acids and octadecanoic acids were detected in the leaf extract by Gas Chromatography-Mass Spectroscopy. White blood cells, hemoglobin and platelets were some of the hematological indices increased after the oral administration of the extract. Red cell distribution width (RDW), a platelet index implicated in cardiovascular ailments was lowered after consumption of the leaf extract of *C. albidum*. The liver marker enzymes aspartate transaminase and alanine transaminase showed no significant changes in their serum activity. Similarly, superoxide dismutase and catalase showed no significant increase after the period of study.

Article Information

Received 03 October 2020

Revised 18 November 2020

Accepted 04 December 2020

Available online 07 May 2021

Authors' Contribution

EBO, OKO designed research. OJN, AOV and OKO carried out laboratory work, wrote manuscript. EBO, UDC and OKO analyzed results and wrote manuscript.

Key words

Platelet indices, Hematological indices, Phytomedicine, *Chrysophyllum albidum*, Enzymes

INTRODUCTION

Chrysophyllum albidum is common tree found in Africa, known for its edible fruits and medicinal values (Amusa *et al.*, 2003). It is predominant in rainforests, which can reach 25-37m when matured (Orwa *et al.*, 2009). It is known commonly in Nigeria as Agbalumo and Udara among the Yorubas and Igbos. It is rich in phytochemicals such as alkaloids, phenols and tannins (Okoli and Okere, 2010), alkaloids and tannins (Ibrahim *et al.*, 2017). Imaga and Urua (2013) and Egharevba *et al.* (2015) reported that the plant is rich in phytochemicals.

The plant is used to cure yellow fever, malaria, treatment of infections and skin eruptions (Idowu *et al.*, 2006). Similarly, the stem, seeds and root extracts of *C. albidum* exhibit antimicrobial, anti-inflammatory, anti-diarrheal and anti-haemorrhoidal properties (Okoli and Okere, 2010). These pharmacological activities are elicited by phytochemicals they contain (Omeje *et al.*, 2014), it is a remedy for fever due to alkaloids present and the flavonoid is responsible for anti-allergic, anti-microbial and anti-cancer activity (Adisa, 2000). There are several

side effects associated with the usage of phytomedicine in the treatment of ailments, such as deleterious effect on blood (Esonu *et al.*, 2001), liver and kidney (Stickel *et al.*, 2005).

Different parts of *Chrysophyllum albidum* plant (leaf, stem-bark and root extracts) have been employed in ethno-medicine as antimicrobial agent (Okoli and Okere, 2010), fertility enhancer (Oigbochie *et al.*, 2019) and antimalarial agent (Odeiran *et al.*, 2020). Though, the choice of preparation method depends on the type of ailment targeted, plant extract could be taken as a tonic (Morton, 1987), boiled or using local gin.

Despite all the medicinal benefits attributed to the different parts of *C. albidum* plant, some individual reports on dizziness and other allergic conditions after ingesting the trado-medicine have appeared. For these reasons it becomes important to evaluate the ethanol *C. albidum* leaf extract on the biochemical indices of Wistar rats.

MATERIALS AND METHODS

C. albidum leaf was harvested from its natural habitat Edem-ani, Nsukka LGA, Enugu State of Nigeria in the month of March, 2019. After fourteen days drying, it was pulverized into fine powder using electric blender. The ground leaf material (773.760 g) was extracted by

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0030-9923/2021/0001-0001 \$ 9.00/0
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macerating in 1.2L of 70% ethanol for 48 h and concentrate, yielding 18.48 g of the extract stored for further use.

Qualitative and quantitative phytochemical analysis

Qualitative analysis of the phytochemicals was determined using AOAC (2010) method with little modification. The phytochemicals screened were alkaloids, flavonoids, tannins, saponin, terpenoid and steroids.

The phenolic content was determined using the method described by Nwidi *et al.* (2017). The total flavonoid, alkaloid and tannin content of the leaf extract were determined according to Senguttuvan *et al.* (2014), using quercetin as the reference compound.

Gas chromatography-mass spectroscopy (GC-MS) analysis of the ethanol extract

GC-MS analysis was done using GC system comprising a Gas Chromatograph interfaced to a Mass Spectrometer (Schimadzu GCMS-QP2010).

Experimental animal

Twenty-five (25) adult Wistar albino rats (98-168 g) were used for the study. They were housed Biochemistry Department Animal House, and fed with commercial rat chow. The animals were divided into 5 groups, each of five rats. They received the extract orally for twenty-eight days as follows: Group I: 2 ml of distilled water/kg body weight per day; Group II: 50 mg/kg b.w. of ethanol extract per day; Group III: 100 mg/kg b.w. of the ethanol extract per day; Group IV: 150 mg/kg b.w. of the ethanol extract per day; Group V: 200 mg/kg b.w. of the ethanol extract per day.

On day 28, blood samples were drawn by cardiac puncture; chloroform was used as a sedative agent. Each container was thoroughly mixed with anticoagulant (3.8% Tri-sodium-citrate) to prevent coagulation of the blood.

Haematological indices

The blood samples were drawn from the animals (control) at the start of experiment and then on the 28th day for determination of haematological parameters such as hemoglobin, white blood cells, haemocrit, red blood cells, platelet concentration, mean cell volume and lymphocyte concentration, platelet indices (red cell distribution width standard deviation, red cell distribution width coefficient of variation, platelet distribution width, mean platelet volume, platelet large cell ratio) by an Automated Hematology Sysmex Analyzer (Coulter Electronics, Bedfordshire, England).

Biochemical components of blood

Superoxide dismutase activity was determined using

Martin *et al.* (1987) method. Alanine aminotransferase and aspartate aminotransferase activities were measured as described by Reitman and Frankel (1957). Catalase activity was determined as described by Aebi (1984) by monitoring the ultraviolet absorption of the sample at 240 nm as hydrogen peroxide is decomposed in by catalase.

Statistical analysis

The statistical packaged used was the statistical package for social sciences (SPSS), version 1. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

C. albidum was harvested from Edem-ani community, its natural habitat and macerated for 48 h. The extract yield was 18.48 g. Alkaloids, tannins, saponins, terpenoids and flavonoids were detected, while steroids was not detected. The result agreed with the report of Oputah *et al.* (2016), who reported saponins and flavonoids, while tannins, alkaloids, terpenoids, saponins, reducing sugars, steroids and flavonoids were reported by Akinpelu *et al.* (2016) in their study on *C. albidum* stem bark extract. It is evident that the plant parts of *C. albidum* are rich source of phytochemicals, which could be responsible for the reported pharmacological activities. Similarly, Oguntoyinbo *et al.* (2015) reported flavonoids, tannins, terpenoids and cardiac glycoside in the leaf of *C. albidum*.

Table I shows the concentration of alkaloids (0.415%), tannins were (0.102 g/TAEq), total flavonoids (0.211 mg/QEq) and total phenolic (0.360 mg/GAEq). Total phenolic and flavonoid content were high when compared to 68.3 µg GAE/g and total flavonoid of 3.71 mg reported by Asare *et al.* (2015). Also, low total phenol content and total flavonoid content of 0.47mg/gQE were reported by Oguntoyinbo *et al.* (2015). The total flavonoid content of *C. albidum* leaf extract was low, when compared to 1.79 mg/gQE reported for *Euphorbia neruifolia* leaf extract (Pracheta *et al.*, 2010). The antimicrobial property may be attributed to the presence of phenolic compounds. This result indicates that the leaf extract could possess antioxidant activity due to the presence of polyphenols. Some pharmacological activities attributed to alkaloids include antimicrobial, cytotoxic, antioxidant, antimutagenic, and hallucinogenic properties (Patel *et al.*, 2012). Tannin is important because of its ability to mop up free radicals (Deng *et al.*, 2019), that can bind and precipitate or sink proteins (Ashok and Upadhyaya, 2012).

Figure 1 shows Gas Chromatography-Mass Spectrometry analysis (GC-MS) (Shibula and Velavan, 2015) of the ethanol extract of *C. albidum* which shows eight peaks. The corresponding compounds identified are

shown in Table II. n-Hexadecanoic acid is an important biocidal agent. It has hypo-cholesterolemic and antioxidant properties (Komansilan *et al.*, 2012). Phytol has been reported to significantly reduce the parasitic load of human schistosomiasis (Moraes *et al.*, 2014). GC-MS was employed in studying the bioactive components of *Physalis minima* leaves (Karpagasundari and Kulothungan, 2014).

Table I. Quantitative phytochemical composition of ethanolic leaf extract *C. albidum*.

Phytochemicals	Composition
Alkaloid content	0.415 %
Tannin	0.102 mg/TAEq
Total Flavonoid content	0.211 mg/QEq
Total phenolic content	0.360 mg/GAEq

Table II. Compounds identified by GC-MS from ethanol extract of *C. albidum*.

S. No.	Name of compound	Conc. (Area %)	Retent. time (s)	Mol. formular
1	Pentadecanoic acid	1.72	19.32	C ₁₇ H ₃₄ O ₂
2	n-hexadecanoic acid	19.86	19.97	C ₁₆ H ₃₂ O ₂
3	11, 14, 17 eicosatrienoic acid	1.33	21.79	C ₂₁ H ₃₆ O ₂
4	Trans-phytol	2.92	22.08	C ₂₀ H ₄₀ O
5	Oleic acid	56.40	22.77	C ₁₈ H ₃₄ O ₂
6	Octadecanoic acid	9.42	23.15	C ₁₈ H ₃₆ O ₂
7	Hexadecanoic acid	1.70	25.16	C ₁₉ H ₃₈ O ₄
8	9-Octadecenal	6.65	27.44	C ₁₈ H ₃₄ O

Table III shows that the oral administration of the extract had no significant impact on the red blood cell count, while the white blood cell increased significantly when compared with the control. At 200 mg/kg bwt of the extract, the hemoglobin concentration increased significantly when compared to the control. Also, PCV and MCHC reduced significantly when compared to the control. The values of lymphocytes and neutrophil did not show any significant change when compared with the control. This suggests that, the leaf extract of *C. albidum* contain some important pharmacological agents responsible for impairment in the production of blood cells (PCV and MCHC) (Adebayo *et al.*, 2011). The changes observed in some of the indices are an indication that some of the phytochemicals present have impact on blood indices synthesis and morphology. Tannin and alkaloids have been reported to cause changes in blood

parameters (Deng *et al.*, 2019). Similarly, the mechanism of antimicrobial property of *C. albidum* leaf extract could be by increasing the concentration of the white blood cells, thereby mobilizing them to the site of infection. Omeje *et al.* (2014) had reported the alteration of blood indices by the leaf extract of *C. olitorus*. The significant increase in the values of platelet at all doses suggests that *C. albidum* may be of great importance to an individual who is to under cesarean section in order to help boost platelet aggregation, thereby leading to wound healing.

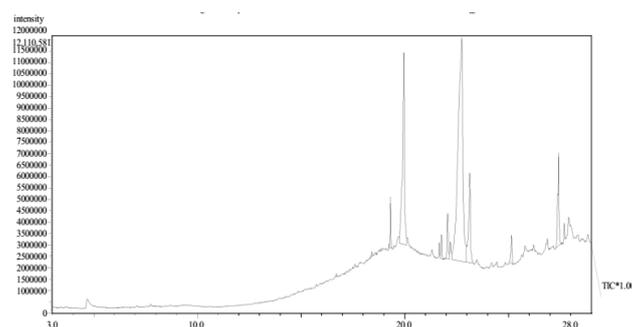


Fig. 1. GC-MS spectra of the leaf extract of *C. albidum*.

Table IV shows the effect of leaf extract on platelet indices. There was significant decrease of RDW-CV and PDW when compared to control group. A dose dependent decrease of the RDW-CV concentration was observed. The concentrations of MPV and P-LCR and RDW-SD increased significantly when compared with the control group. This is in accordance with the findings of Ofem *et al.* (2012) where doses of plant extract caused decrease in MPV, P-LCR and PDW of albino rats. This suggests the ethanol extract of *C. albidum* does not contain bioactive compound that affects the tested parameters. One of the indicators of cardiovascular mortality is increased red cell distribution width (RDW) (Söderholm, *et al.*, 2015). This suggests the consumption of *C. albidum* leaf extract may not be predisposing a consumer to any cardiovascular illness. According to Tonelli *et al.* (2008), increased RDW is implicated in long-term mortality of patients suffering from coronary artery disease. Hence, the decrease observed, shows that the ethanol extract of *C. albidum* leaf may not cause or induce cardiovascular diseases by platelet index increase. Similarly, iron deficiency anemia has been linked with increased RDW (Khan *et al.*, 2014). The non-significant increase in RDW shows that the extract may not have deleterious effect or inhibit red blood cell synthesis. Presence of tannin in plant extract has been implicated in the alteration of platelet indices (Muriithi *et al.*, 2015).

Aspartate aminotransferase (AST) and alanine

Table III. Effect of *C. albidum* leaf extract effect on haematological indices of albino rats.

Hematological parameter	Control	50 mg/kg bwt	100 mg/kg bwt	150 mg/kg bwt	200 mg/kg bwt
White blood cell ($\times 10^9/L$)	5.28 \pm 1.29	5.35 \pm 0.14	7.41 \pm 1.62	7.21 \pm 0.22	9.05 \pm 0.13
Red blood cell ($\times 10^{12}/L$)	5.11 \pm 1.77	5.20 \pm 1.31	6.45 \pm 0.31	5.34 \pm 0.40	6.09 \pm 0.62
Hemoglob. (g/dl)	6.72 \pm 0.43	6.53 \pm 0.83	8.64 \pm 0.92	6.07 \pm 0.11	8.35 \pm 1.03
HCT (%)	32.17 \pm 0.38	23.91 \pm 1.65	31.60 \pm 0.52	24.31 \pm 1.46	33.24 \pm 0.29
PCV (fl)	66.24 \pm 2.46	45.72 \pm 2.52	48.14 \pm 3.87	49.74 \pm 2.38	44.04 \pm 4.23
MCHC (pg)	22.75 \pm 0.18	21.92 \pm 0.59	19.20 \pm 0.53	19.73 \pm 1.26	19.60 \pm 0.22
Platelet ($\times 10^9/L$)	131.73 \pm 63.34	147.72 \pm 11.07	129.66 \pm 21.40	301.80 \pm 0.74	344.46 \pm 4.23
Lymphocyte (%)	68.29 \pm 0.56	69.85 \pm 0.62	66.39 \pm 2.81	74.27 \pm 3.70	70.04 \pm 0.28
Neutrophils (%)	25.42 \pm 1.37	24.72 \pm 0.56	30.44 \pm 0.41	33.63 \pm 1.64	23.89 \pm 3.22

Table IV. Effect of oral administration of ethanol extract of *C. albidum* on platelet indices of wistar rat

Platelet indices	Control	50 mg/kg bwt	100 mg/kg bwt	150 mg/kg bwt	200 mg/kg bwt
RDW-CV (%)	20.73 \pm 1.92	16.22 \pm 1.50	14.38 \pm 0.80	13.85 \pm 0.62	13.42 \pm 0.00
PDW (fL)	9.94 \pm 0.41	5.42 \pm 0.46	3.76 \pm 0.63	5.11 \pm 1.28	7.93 \pm 2.06
MPV (fL)	-9.27 \pm 0.77	5.29 \pm 0.31	-6.83 \pm 0.40	-9.61 \pm 0.43	-8.63 \pm 0.10
P-LCR (%)	4.65 \pm 0.43	9.42 \pm 0.38	5.61 \pm 0.92	7.52 \pm 0.10	7.16 \pm 0.53
RDW-SD (fL)	27.59 \pm 0.28	27.65 \pm 0.90	25.40 \pm 0.65	22.65 \pm 0.00	19.34 \pm 0.44

RDW-CV, red cell distribution width coefficient of variation; PDW, platelet distribution width; MPV, mean platelet volume; P-LCR, platelet large cellratio; RDW-SD, red cell distribution standard deviation.

Table V. Effect of oral administration of ethanol extract of *C. albidum* on representative liver function and oxidative stress related enzymes in wistar rats.

Biochemical indices	Control	50 mg/kg bwt	100 mg/kg bwt	150 mg/kg bwt	200 mg/kg bwt
Alanine transaminase (IU/L)	116.05 \pm 0.43	113.92 \pm 0.30	109.20 \pm 0.42	132.09 \pm 0.18	193.22 \pm 0.12
Aspartate transaminase (IU/L)	184.76 \pm 0.26	184.45 \pm 0.18	139.28 \pm 0.30	170.58 \pm 0.07	186.41 \pm 0.25
Catalase (IU/L)	194.50 \pm 0.49	207.53 \pm 0.32	171.06 \pm 0.61	193.41 \pm 0.70	176.39 \pm 0.10
Superoxidismutase (IU/L)	202.70 \pm 0.90	200.65 \pm 0.25	186.14 \pm 1.30	185.29 \pm 0.05	213.12 \pm 0.29

aminotransferase (ALT) are some enzymes considered as indicators of the health of the liver (Trampuz *et al.*, 2003). Table V shows effect of varying concentrations (50, 100, 150 and 200mg/kg) of extract of *C. albidum*. Increase in serum ALT is common in hepatic necrosis (Duncan *et al.*, 2006). At concentrations of 150 and 200 mg/kg body weight, there was significant increase in the serum ALT. The observed increase suggests the low concentration of free radicals in the biological system, which could be attributed to the ability of some bio active agents to mop up these free radicals or maintaining the integrity of the hepatocytes to produce adequate enzymes needed to play the antioxidant role. Omeje *et al.* (2016) reported hepatoprotective potential of ethanol extract of *C. olitorus*. AST increase in serum signifies liver damage (Dasofunjo

et al., 2013). The oral administration of the extract showed a similar result to *Buchholzia coriacea* extract on *Plasmodium berghei* infected mice as reported by Enechi *et al.* (2016). Therefore, the significant decrease obtained suggests the extract has the ability to protect the integrity of the animal liver.

Superoxide dismutase (SOD) is an enzyme responsible for scavenging free radicals for physiological defense strategies in living organisms (Stephanine *et al.*, 2020). Varying concentration of *C. albidum* ethanol extract has shown a significant decrease when compared to control group. The results show a dose dependent decrease in the activity of SOD except for 200 mg/kg group. This is suggestive that high concentration of free radical was generated in vivo, hence the decreasing

concentration of the enzyme responsible its mop up. This report is in line with the study by [Owolabi et al. \(2008\)](#). Similarly, [Nwobodo et al. \(2018\)](#) reported decreased SOD in their work on *Azadirachta indica*. Catalase is one of the antioxidant enzymes that plays essential role in the antioxidant protection biological systems ([Ighodaro and Akinloye, 2018](#)). Among the concentrations studied, there was no significant difference when compared to the control. Suggesting that, the rate of generation of free radical was in equilibrium with the rate of its clearance. This could be attributed to the presence of polyphenolic compounds in the ethanol extract. [Sani et al. \(2012\)](#) reported increased concentration of catalase and superoxide dismutase in their work on three medicinal plants. There is synergistic relation between catalase and superoxide dismutase, since they are the first line of body defense against oxidative stress ([Ighodaro and Akinloye, 2018](#)).

CONCLUSION

In conclusion, the oral administration of *C. albidum* leaf extract did not cause significant alteration in the hematological and platelet indices (RDW, PDW, MPV and P-LCR) at the doses studied after 28 day consumption. One of the indicators of cardiovascular mortality is increased red cell distribution width (RDW), which was reduced by the ethanol extract. This suggests that the consumption of *C. albidum* leaf extract may not be predisposing a consumer to any cardiovascular illness. This suggests therefore, there is no harmful effect associated with the consumption of *C. albidum*, however, having established the preservatory property of the *C. albidum* extract on healthy models, further studies could be carried out on diseased conditions to ascertain if the extract is curative.

Statement of conflict of interest

The authors have declared no conflict of interest.

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