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Standardization, Chemical Composition and Antipyretic Evaluation of the Methanol Leaf Extract and Fractions of *Chrysophyllum albidum* (Sapotaceae)

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ABSTRACT

Alternative antipyretic agents are needed to circumvent the toxicities associated with current agents. *Chrysophyllum albidum* leaves are used ethnomedicinally in pyrexia treatment. There is a need to evaluate this claim scientifically, its composition and establish its pharmacognostic standards. In this study, a 1 kg of dried pulverized *C. albidum* leaves was cold macerated in methanol for 72 hours and evaporated *in vacuo*. Approximately 40 g of this extract was reconstituted and fractionated exhaustively using n-hexane, ethyl acetate, butanol, and water successively to obtain the respective fractions. The extract was subjected to acute toxicity, proximate, phytochemical and antipyretic evaluations. The fractions were also evaluated for antipyretic activity. The extract and fractions were also subjected to HPLC analysis. The LD_{50} was 2739 mg/kg. The leaf has low ash value (8.50%), moisture content (4.70%) and water extractive value (15.13%). The leaf extract contained trace alkaloids (2.2%) and tannins (1.13%); with moderate flavonoids (10.6%) and saponins (13.65%). The antipyretic study revealed that the 500 mg/kg of methanol extract and butanol fraction had mild antipyretic activity. The HPLC analysis suggested the presence of nigricinol, dinonedimethoxyketal, indo-3-carbaldehyde, isorhamnetin-3-O-retinol, 1-hydroxy-3, 4-dihydronorharmane, pestalotiolactone A and nidulalin B from methanol extract. Presence of citrinin hydrate and pestalotiolactone A was suggested in the n-hexane fraction; expansol B and citreonigrin E in the ethyl acetate fraction and septicine in the butanol fraction. The study results support the ethnomedicinal use of *C. albidum* leaves for fever treatment.

***Keywords*:** Antipyretic, Fever**,** *Chrysophyllum albidum*, Toxicity, Proximate analysis.

Introduction

Pyrexia, also known as fever and febrile response is an increase in body temperature (when the body temperature goes up above the normal range of 36.5–37.5°C (97.7–99.5°F))[1] due to an increase in the body temperature set point.[2] It is a temporary increase in body temperature, often due to illness. Fever can be caused by many medical conditions such as viral, bacterial, and parasitic infections including the common cold, urinary tract infections, meningitis, malaria and appendicitis among others. Non-infectious causes include vasculitis, deep vein thrombosis, side effects of medication, and cancer among others.[2] Fever is one of the most common medical signs. It contributes to the approximately 30% of healthcare visits by children[3] and occurs in up to 75% of adults who are sick.[4] Our immune systems attempt to combat infection by elevating body temperature.[4,5] Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin or ibuprofen, antibiotics and fluid intake can help reduce fever.[5]

Chrysophyllum albidum, commonly known as white star apple, belongs to the family of *Sapotaceae*. In Nigeria, it is known by various local names such as *Agbalumo* in Yoruba, *Udara* in Igbo and *Agwaluma* in Hausa.[6-8] It is a forest tree species that usually grows up to 25 to 37 m in height.[6] It was described as a forest fruit tree by the Scottish botanist George Don.[8] It is found throughout the tropical, Central, East and West Africa regions and other parts of the world.[9, 10] The fruit as shown in Figure 1 is ovoid to sub-globose, pointed at the apex, and up to 6 cm long and 5 cm in diameter, the skin or peel, is orange to golden yellow when ripe and the pulp within the peel maybe orange, pinkish, or light yellow when it is ripe. About three to five seeds, as shown in Figure 1, are found within the pulp and are not usually eaten. The seed-coats are hard, bony, shiny, and dark brown, and when broken reveals white-coloured cotyledons. It is a seasonal fruit (usually from December to March). However, the plant is a crop of commercial value in Nigeria.[11] *Chrysophyllum albidum* is used for various medicinal purposes in ethnomedicine. The bark is employed for the treatment of yellow fever and malaria in folklore medicine.[8] Its leaves are used as emollient and for the treatment of malaria, stomach ache, and diarrhea. Also, its leaves and cotyledons from its seed are used as ointments in the treatment of vaginal and dermatological infections in Western Nigeria.[11] The seeds and roots extracts of *C. albidum* are used to arrest bleeding from fresh wounds, and to inhibit microbial growth of known wound contaminants and also enhance the wound healing process as they have astringent characteristics.[9]

There is a need for an alternative source of antipyretic agents to circumvent the toxicities associated with current agents. The main objective of the study is to determine the potential antipyretic activity of the methanol leaf extract and fractions of *C. albidum on ye*ast induced pyrexia in rats.

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Figure 1: *Chrysophyllum albidum* leaves, fruit and seeds

Materials and Methods

Drug and Solvents

Analytical grades of n-Hexane (JHD, Guangdong), Ethyl acetate (JHD, Guangdong), Methanol (JHD, Guangdong) and Butanol (JHD, Guangdong) were used. Freshly distilled water was used when required. Paracetamol tablets (Emcap®, Emzor, Nigeria) and Baker's yeast (STK International LTD, China) was used.

Equipment

These include oven (CostabSci – tech, China), hot plate (Jenway, UK), rotary evaporator (Balloworld Scientific Limited, UK), vacuum pump (GEC electromotors, UK), furnace (Vestor furnace, Germany), weighing scale (Ohaus, China), UV/visible spectrophotometer, Jenway) and Dionex P580 HPLC system coupled to photodiode array detector (UVD340S, Dionex Softtron GmbH, Germany). Detection was at 235, 254 and 340 nm.

Plant material

Fresh leaves of *C. albidum* were collected from its natural habitat at Adagbe Avomimi village, Enugwu-ukwu, Njikoka Local Government area of Anambra State, Nigeria in August 2015. It was identified by a senior technologist in Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka. A voucher specimen PCG474/A/043 was deposited at the Herbarium of the Department of Pharmacognosy and Traditional Medicine, of Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka.

Animals

Rats (150 – 200 g) and Swiss albino mice (25 – 30 g) were used for the study. The animals were obtained from the animal house of Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka. They were allowed access to food and water *ad libitum*. All the animal experiments were conducted in compliance with National Institute of health (NIH) guide for care and use of laboratory animals.[12]

Determination of analytical standards

The procedure was used to determine the water soluble extractives, total ash, acid insoluble ash, water soluble ash and water loss on drying.[13]

Preparation of plant material

The plant material was cleaned of earthy impurities and air dried for 3 weeks and then ground to powder using a dry laboratory electric milling machine. The powdered material was stored in air-tight container before use.[14]

Extraction and fractionation of plant material

The powdered plant material (1.0 kg) was cold macerated in 3 L of methanol for 72 h with intermittent shaking. The resulting solution was filtered using Whatman's No 1 filter paper and the filtrate concentrated using rotary evaporator at 40^{o}C. A solid residue was obtained and referred to as the crude extract. Then 40 g of the extract was subjected to liquid-liquid partitioning using n-hexane, ethyl acetate, butanol and distilled water in order of their increasing polarity. The fractions were filtered and concentrated using rotary evaporator at 40^{o}C.[15]

Phytochemical screening of the powdered leaf

Qualitative phytochemical analysis was determined using standard procedures. The phytoconstituents evaluated include alkaloids, glycosides, steroids, flavonoids, saponins, tannins, terpenes, proteins, carbohydrates, and reducing sugars. The leaf powder of *C. albidum* was also subjected to quantitative phytochemical evaluation of alkaloids, tannins, flavonoids and saponins.[16-18]

Acute toxicity test

Acute toxicity of the leaves extract was carried out using 32 mice grouped into eight groups A-H (n = 4). Groups A, B, C and D received 100 mg/kg, 500 mg/kg, 1000 mg/kg and 2000 mg/kg of methanol extract of *C. albidum* leaf, respectively, while groups E, F and G received 3000 mg/kg, 4000 mg/kg, and 5000 mg/kg of methanol extract of *C. albidum* leaf, respectively. Group H received 5 mL of distilled water as a control. The extract was dissolved in distilled water and was orally administered to the different groups after an overnight fast. The number of deaths in each group within 24 h was recorded. The animals were left for further 14 days for any delayed signs of toxicity.[19]

Antipyretic study

In the antipyretic screening, pyrexia was induced using baker's yeast. Fifty-four albino rats grouped into nine groups A - I (n = 6) were used for the study. Groups A, B, C, received 125, 250 and 500 mg/kg of methanol leaf extract of *C. albidum*, respectively, groups D, E, F and G received 500 mg/kg n-hexane fraction, 500 mg/kg ethyl acetate fraction, 500 mg/kg butanol fraction, and 500 mg/kg aqueous fractions of *C. albidum* leaf, respectively, group H received 10 mg/kg paracetamol as a positive control, while group I received 5 mL of distilled water as a negative control. The animals were administered baker's yeast subcutaneously and 18 h later, rectal temperatures of the rats were measured by inserting a thermometer into the rectum of the animals. After 1 h, *C. albidum* methanol extract, *C. albidum* fractions, paracetamol (both dissolved in distilled water) and an equivalent volume of distilled water (negative control) were administered to the

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respective groups. The rectal temperatures of the animals were subsequently measured at 30, 60, 90 and 120 min post methanol extract, fractions, drug and distilled water administration. The mean of post baker's yeast rectal temperatures was compared with pre-drug treatment temperature.[20]

HPLC analysis
The extract and fractions were also subjected to HPLC analysis. 2 mg each of the extract and fractions were reconstituted with 2 mL of HPLC grade ethanol, using Dionex P580 HPLC system coupled to photodiode array detector (UVD340S, DionexSofttron GmbH, Germany). Detection was at 235, 254 and 340 nm[14].

Statistical analysis
Data obtained from the study were expressed as the mean ± SEM. Statistical comparisons between the groups were made using the one-way analysis of variance (ANOVA). The level of significant difference between the groups was evaluated at $P < 0.05$.

Results and Discussion

Qualitative and quantitative phytochemical screening
The results of qualitative and quantitative phytochemical analyses of *C. albidum* leaves are presented in Tables 1 and 2, respectively.
The result of the phytochemical screening of *C. albidum* revealed that the methanol extract contains alkaloids, tannins, cardiac glycosides, carbohydrates, flavonoid, saponins, reducing sugar and proteins; however, steroids and terpenoids were absent. The n-hexane fraction contains saponins, cardiac glycosides, carbohydrate, while alkaloids, tannins, reducing sugars, steroids and terpenoids were absent. The ethyl acetate fraction contains alkaloids, cardiac glycosides, carbohydrates, tannins, saponins, proteins, while reducing sugars, steroids and terpenoids were absent. The butanol fraction contains tannins, reducing sugars, flavonoids, saponins, cardiac glycosides, protein, while alkaloids, carbohydrates, steroids and terpenoids were absent. The aqueous fraction contains trace alkaloids, tannins, saponins, reducing sugars, carbohydrates, proteins, while flavonoids, cardiac glycosides, steroids and terpenoids were absent. The methanol extract result was similar to what Idowu *et al.* and Ushie *et al.* reported.[21, 22]
The quantitative analysis revealed that *C. albidum* contains trace amount of alkaloids (2.20%) and tannins (1.13%), moderate amount of saponins (13.65%) and flavonoids (10.6%). The medicinal value of plants lies in some chemical substances that have definite physiological actions. The phytochemicals may act as precursors for bioactive compounds used as therapeutic drugs. Different phytochemicals have been found to possess a wide range of activities which may help in protection against pyrexia. Flavonoids have been reported to exhibit antipyretic effect.[23] Plants with phytochemicals such as anthraquinones, saponins and alkaloids have been reported to have the potential to exhibit antimalarial activity.[24]
Furthermore, saponins have been reported to possess antiprotozoal activity and this characteristic has been studied using animals.[25, 26] The presence of most general phytochemicals might be responsible for the antipyretic effect.

Proximate analysis of leaves of C. albidum
The result of the proximate analysis of the leaves of *C. albidum* is presented in Table 3. The proximate analysis of *C. albidum* leaf extract showed the following quantitative parameters; Total ash 8.50%, Acid insoluble ash 3.91%, Water soluble ash 2.94%, Water extractives 15.13%, and water Loss on drying/Moisture content 4.70%.
The physico-chemical parameters are helpful in judging the purity, quality and shelf-life of crude drugs.[16, 27-29] The ash value of any organic matter is composed of their non-volatile inorganic component. This value may vary from plant to plant hence an important parameter in the evaluation of crude drugs. The ash value may be raised by unwanted parts of drug and contaminants such as sand.[30] Estimation of ash values is also a significant parameter for the detection of the nature of material, which is added to the drug for the purpose of adulteration, impurities and determination of authenticity, quality and purity of test sample.[31]
Lower content of total ash in this result as compared to European Pharmacopeia specification of dried leaves of less than 16% indicates low level of carbonates, phosphates and silica in the leaves of *C. albidum*. Results of acid insoluble ash and water soluble ash showed low content of acid insoluble ash and water soluble ash in the leaves of *C. albidum*, compared to European Pharmacopoeia specification of dried leaves of less than 4% of acid insoluble ash, indicating low level of carbonates, phosphates and silica in the leaves of *C.* albidum.[28, 30, 32]
Extractives are the number of active constituents in a given amount of medicinal plant material when extracted with solvents. Water soluble extractives value indicates the presence of sugar, acids and inorganic compounds, thus, may be useful in detection of adulteration whether deliberate or not and to indicate poor quality of drugs.[28, 30] The result from this study may suggest that the dried leaves of *C. albidum* have high water soluble extractive value. This may be indicative of the presence of water-soluble matter such as alkaloids, carbohydrates and flavonoids. These organic matters may possess some therapeutic effects which may be used to develop potential drugs.
Determination of moisture content helps in the estimation of the amount of volatile matter, this is very critical and the monographs specified limit to ensure a high quality of such drugs. Most drugs may be stored safely if the moisture content is kept as low as 6% or less.[3, 26, 31, 32] However, the rate at which the moisture is removed and the conditions under which it is removed can influence the moisture content. Higher moisture content indicates higher humid condition of the extract, which favours the growth of fungi or may cause other micro-organic contamination that may result in deterioration of the drug. The results from this study indicate that the plant material has low moisture content as compared to the BP specification as earlier stated. Hence the dried leaves of *C. albidum* may be safely stored.

Table 1: Phytochemical screening of *C. albidum* leaves

Constituent	Methanol extract	N-hexane fraction	Ethyl acetate fraction	Butanol fraction	Aqueous fraction
Alkaloids	+	-	+	-	+
Tannins	+	-	+	+	+
Flavonoids	+	-	+	+	-
Saponins	+	+	+	+	+
Cardiac glycosides	+	+	+	+	-
Reducing sugar	+	-	-	+	+
Carbohydrates	+	+	+	-	+
Proteins	+	+	+	+	+
Steroids	-	-	-	-	-
Terpenes	-	-	-	-	-

Key: + Present and - Absent.

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Table 2: Quantitative analysis of leaves of *C. albidum* leaves

Phytochemical	Percentage content (%) ± SEM
Alkaloids	2.20 ± 0.43
Saponins	13.65 ± 1.21
Flavonoids	10.6 ± 0.89
Tannins	1.13 ± 0.57

The values represent mean ± SEM (n = 3)

Table 3: Proximate analysis of leaves of *C. albidum* (physico-chemical parameter)

Parameter	Mean Values (%) ± SEM
Total ash	8.50 ± 0.27
Acid insoluble ash	3.91 ± 0.20
Water soluble ash	2.94 ± 1.40
Water extractives	15.13 ± 0.18
Water Loss on drying/ Moisture content	4.70 ± 0.03

The values represent mean ± SEM (n = 3)

Acute toxicity test

In the acute toxicity test, one death was recorded in the groups treated with doses from 3000-4000 mg/kg b.w of *C. albidum* leaf. The oral LD_{50} of the plant extract in mice was calculated to be 2739 mg/kg b.w. The result of the LD_{50} signifies that the plant may be toxic at high doses. The result of this present study is similar to those reported by Ebi and Ofoefule[34] This was necessary as some medicinal plants have shown toxic effects.[35]

Antipyretic activity of the crude extract and fractions of C. albidum leaf

The result of the effects of the methanol leaf extract and fractions of *C. albidum* on baker's yeast-induced pyrexia in rats is shown in Figures 2 and 3.

The results showed that the groups treated with 125 and 250 mg/kg b.w of crude extract, 500 mg/kg b.w n-hexane fraction, 500 mg/kg b.w ethyl acetate fraction and 500 mg/kg b.w. aqueous fraction did not show any significant reduction in rectal temperatures ($P > 0.05$) of the animals throughout the period of this study (120 min) as compared to both positive and negative controls. The groups treated with 500 mg/kg of methanol extract and 500 mg/kg of butanol fraction showed a significant reduction in rectal temperatures ($P < 0.05$). The groups treated with 500 mg/kg of methanol extract and 500 mg/kg of butanol fraction reduced rectal temperatures in the animals. Paracetamol which was used as the positive control also significantly reduced rectal temperatures of the treated animals ($P < 0.05$), throughout the period of observation in this study. Thus, this may indicate that 500 mg/kg of methanol extract, 500 mg/kg of butanol fraction and paracetamol reduced rectal temperatures of the treated animals in a time-dependent manner. This may also indicate that 500 mg/kg of methanol extract and 500 mg/kg butanol fraction of *C. albidum* leaf may show a sustained reduction in rectal temperature if there is a follow up dose after the initial administration. These results showed that *C. albidum* leaf has mild antipyretic activity. The mild antipyretic activity observed may be attributed to the presence of flavonoids, which have been earlier reported to exhibit antipyretic effect.[23]

Furthermore, antipyretics have been shown to suppress fever by inhibiting prostaglandin synthetase, resulting in the blockade of the synthesis of prostaglandin in the brain or suppressing the rise of interleukin-1α production subsequent to interferon production. Flavonoids like baicalin have been shown to exert antipyretic effect by suppressing TNF-α, [2] and its related compounds also exhibit inhibition of arachidonic acid peroxidation, which results in reduction of prostaglandin levels thus reducing the fever and pain.[36]

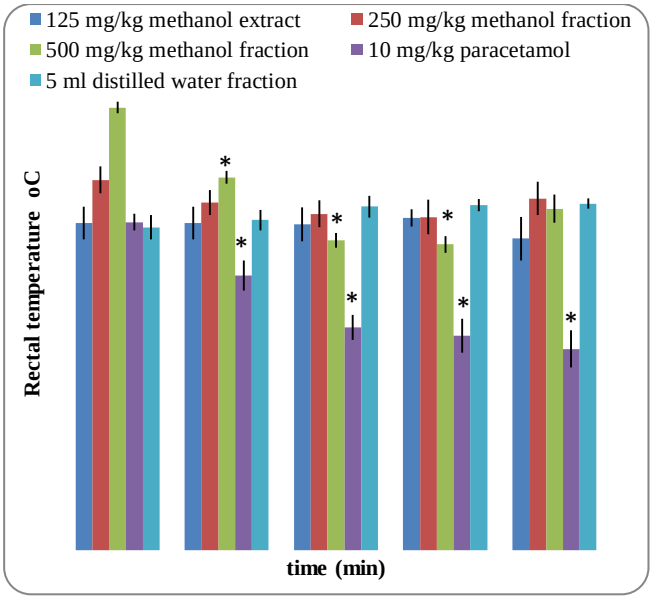

Figure 2: Effects of methanol extract of *C. albidum* on rectal temperatures of yeast induced pyrexia in rats.
Values are expressed as Mean ± SEM. n = 6 in each group, "*" indicate P < 0.05 compared to control.

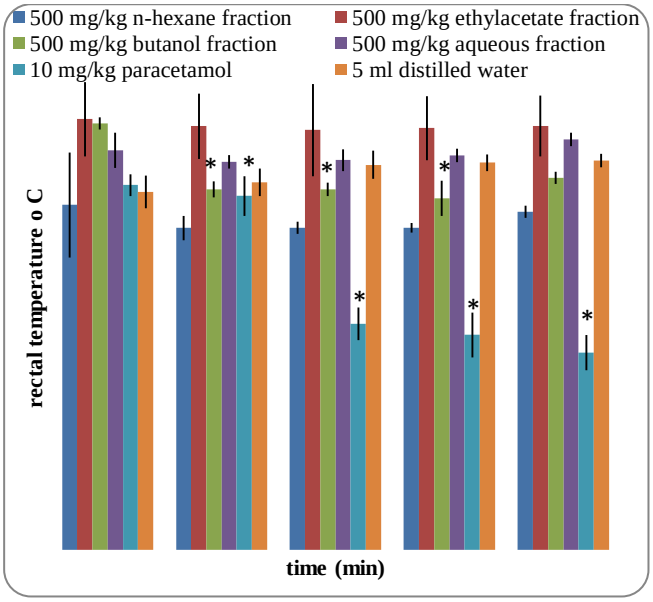

Figure 3: Effects of fractions of *C. albidum* leaves on rectal temperatures of yeast induced pyrexia in rats.
Values are expressed as Mean ± SEM. n = 6 in each group, "*" indicate P < 0.05 compared to control.

The findings from this present study may be due to phyto-compounds like flavonoids and saponins, which are suggested to act synergistically to exert the observed antipyretic activity, [37] as these phytochemicals were found present in *C. albidum* leaf extract.

Five compounds were detected from the methanol leaf extract based on the UV max in the HPLC-DAD analysis. They are Dinonedimethoxyketal, Nigricinol, Indole-3-carbaldehyde, Isorhametin-3-Ortinol and 1-hydroxy-3, 4-dihydronorharman. Citrinin hydrate and Pestalotiolactone were seen in n-hexane fraction in trace amounts while the major peaks with the retention times; 38.90 min, 39.08 min and 39.97 min have no spectra hit which suggests a possibility of new metabolite in *C. albidum* leaf. Expansol B and

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citreonigrin E were seen in ethyl acetate fraction. Indole-3-carbeldehyde, 1-hydroxy-3, 4-dihydronorharman and equietin were among the most prominent peak in the n-butanol fraction. Some phenolics have been reported from the stem-bark of this plant genus *Chrysophyllum*. Also from the seed oil and cotyledons were reportedly isolated eleagnine, tetrahydro-2-methylharman and skatole with anti-microbial activity.[11, 20, 21, 38] Considering the activity reported in this study, the main constituent suggested by HPLC-DAD analysis of the methanol extract and fractions of this plant; the antipyretic activity could be associated with the HPLC-DAD analysis suggested phenolic constituents.

Conclusion

The findings of this study support the use of this plant in the traditional treatment of pyrexia in Nigeria; however the plant has mild antipyretic activity. We recommend further purification of the fractions and detailed confirmation of the HPLC-DAD suggested structures with NMR analysis.

Conflict of interest

The authors declare no conflicting interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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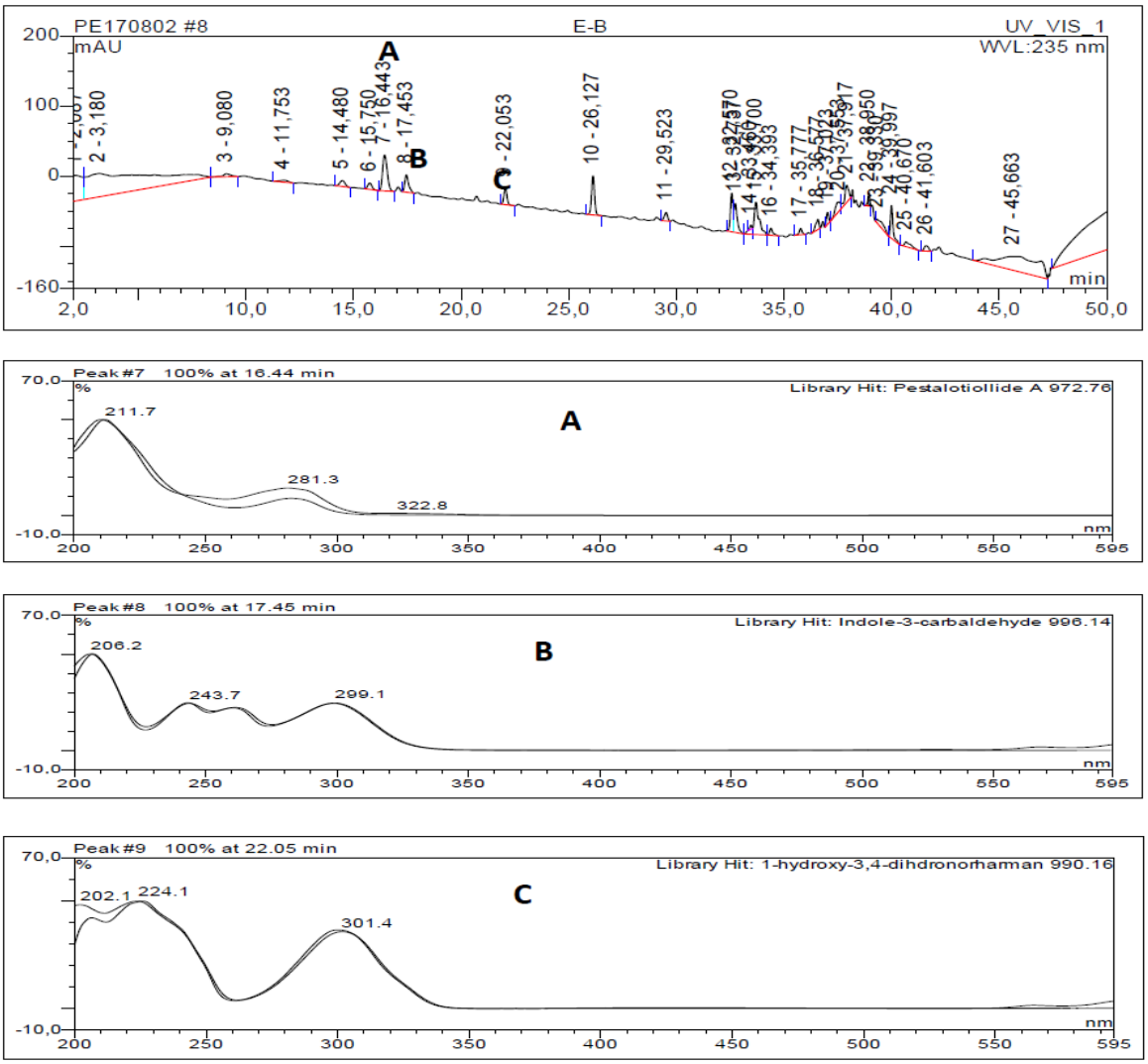

Figure 4: HPLC chromatogram and UV spectra of major compounds detected in the Butanol fraction of leaf extract of *C. albidum* A = Pestalotiolide A (RT = 16.44 min, Hit 998.90), B = Indole-3-carbaldehyde (RT = 17.45, Hit 996.14), and C = 1-hydroxy-3, 4-dihydronorharman (RT = 22.05 min., Hit 990.16).

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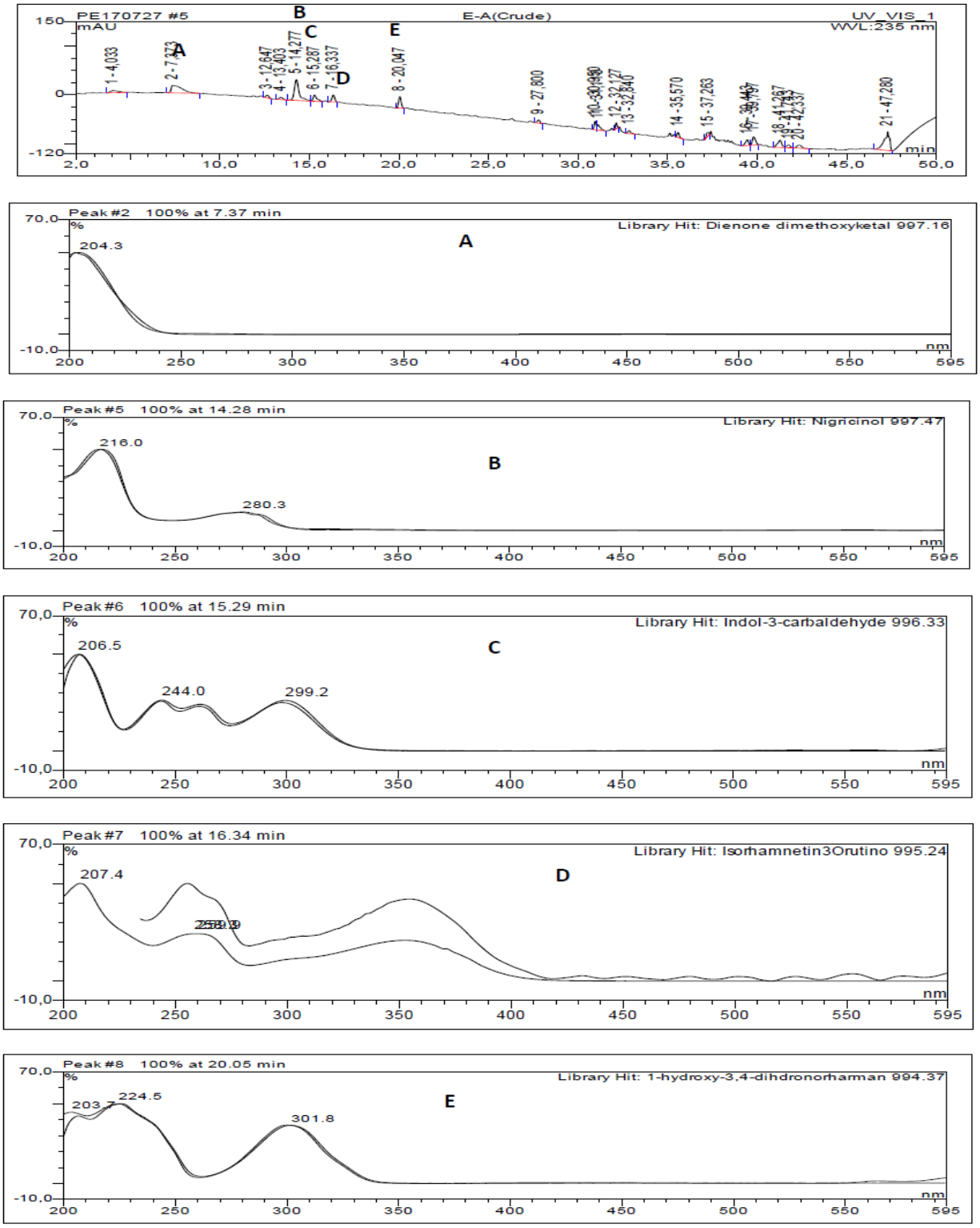

Figure 5: HPLC chromatogram and UV spectra of major compounds detected in the methanolic leaf extract of *C. albidum* A = Dinonedimethoxyketal (Rt = 7.37 min., Hit 997.90), B = Nigricinol (Rt = 14.28 min., Hit 996.14), C = Indole-3-carbaldehyde (Rt = 15.29 min., Hit 996.33), D = Isorhametin3Ortinol (16.34 min., Hit 995.24) and E = 1-hydroxy-3, 4-dihydronorharman (Rt = 20.05 min., Hit 990.16).

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