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Review Article

Crossopteryx febrifuga (Afzel. ex G.Don) Benth; Ethnobotany, Phytochemistry and Pharmacology of an African Tree for Malaria and Beyond

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ABSTRACT

Crossopteryx febrifuga (Rubiaceae) commonly known as the ordeal tree is a single species of the genus and is endemic to Africa with important traditional uses. The review seeks to present an overview of the ethnobotany, phytochemistry and pharmacology of the plant to highlight the significant medicinal benefits of the plant and promote its further development and future exploitation as a phytomedicine. Electronic databases, search engines and botanical web sources were employed in the literature search on the plant with specific search words or keywords used. Traditional medicinal uses of the plant for the treatment of ailments such as cough, fever, pains, malaria, diarrhea, wound infections, abdominal pains and sleeping sickness are reported. In the area of phytochemistry, saponins, terpenes, steroids, flavonoids iridoids and sugar have been isolated from the plant. The extracts and isolated constituents of the plant have demonstrated a wide range of *in vitro* and *in vivo* pharmacological activities including antitussive, anti-inflammatory, antipyretic, antiplasmodial, antihelminthic, antimicrobial activities and toxicity, amongst others. Research gaps of *C. febrifuga* are presented to fully exploit the potentials of this important African plant.

***Keywords*:** *Crossopteryx febrifuga*, Traditional medicine, Malaria, Phytochemistry.

Introduction

The practice of traditional medicine is as old as man and is the more commonly employed strategy for health care delivery in most developing countries. Africa is endowed with a rich floral biodiversity and a plethora of plants have diverse uses in traditional medicine. Apart from being an element of the cultural heritage of the people, traditional medicine is highly patronized in Africa because of the easy access and less cost compared to orthodox/conventional medicine. African traditional medicine is comprised majorly of medicinal plants, which are a proven source of bioactive chemicals.[1] Numerous varieties of medicinal plants growing in West Africa are widely used against many diseases including endemic and complex diseases like malaria, trypanosomiasis, leishmaniasis, asthma, psychosis, hepatitis and cancer.[2] The Rubiaceae is one such therapeutically useful family of flowering plants, with a total of 73 species growing in sub-tropical Africa and distributed into 34 genera.[3] Rubiaceae species are concentrated in warmer and tropical climates around the world, and are characterized by simple, entire and opposite leaves and connate stipules.[3] *Crossopteryx febrifuga* (Afzel. ex G.Don) Benth is the only species of the genus and the specific epithet 'febrifuga' is associated with the ethnomedicinal use of the plant for the treatment of fever.[4] Although *C. febrifuga* is an important medicinal plant endemic to most African countries and used in the treatment of many tropical diseases, knowledge on the plant is not well documented. The review seeks to bring to the fore literature on the ethnobotany, phytochemistry and pharmacology of the African medicinal plant with the aim to its use towards drug discovery and exploitation in health care delivery.

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Materials and Methods

Literature search was carried out using the databases Scifinder, Pubmed, Scopus, search engines Google, Google Scholar, and other web sources such as The Plant List, Kew Botanical Garden and World Agroforestry center. Combinations of keywords used in the web search included but were not limited to "*Crossopteryx febrifuga*", "phytochemistry", "ethnomedicinal use", "traditional use", "pharmacology", "biological activity", "isolated compounds" and "botany". A criterion for inclusion in the study was primary and secondary literature in English language. There was no limit on the period covered by the review. Chemical structures were drawn using ChemBioDraw Ultra 14.0. Thematic areas were summarized in tabular form where necessary.

Results and Discussion

Three thematic areas are presented in this section: (1) ethnobotany, (2) phytochemistry and (3) pharmacological activities of *C. febrifuga*.

Description of C. febrifuga

Crossopteryx is a monospecific African genus with a wide distribution. The generic name was derived from Greek "krossoi" and "pteron" meaning fringed wing and is based on its seed shape.[4] *Crossopteryx febrifuga* (Afzel.) Benth. is a flowering plant in the family Rubiaceae. It is a deciduous savanna tree (Figure 1). The bark slashes grey or dark brown. The young stem is glabrous. The leaves are opposite and elliptic, ovate or obovate and the apex, acuminate. Inflorescence is dense and fragrant and the calyx lobed. The fruits are globose, purple or black.[4]

Geographical Distribution

C. febrifuga is native to Africa and indigenous to Eastern Africa (Burundi, Ethiopia, Rwanda, Sudan, Uganda, Kenya, Malawi, Mozambique, Zambia, Tanzania), Central Africa (Angola, Central African Republic), Western Africa (Burkina Faso, Cameroon, Chad, Congo, Gabon, Gambia, Ghana, Guinea, Guinea-Bissau, Senegal, Sierra Leone, Mali, Benin, Nigeria, Ivory Coast, and Togo) and Southern Africa (Botswana, Caprivi Strip, Zimbabwe) (See Figure 2).

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Figure 1: The fruits (a) stem, leaves and branches (b), and whole plant (c) of *C. febrifuga*

Figure 2: Geographical distribution of *C. febrifuga* in the African continent.[5]

Specimens of the plant are deposited by different African countries with records of herbarium catalogues. The plant is usually found in rock places or termite mounds and sometimes by lakes and stream sides.[5]

Non-medicinal Uses

C. febrifuga is sometimes grown in plantations to provide shade. The plant is used for reclamation as studies have shown that the tree has a high intrinsic fire resistance and a low mortality rate.[6,7] The plant's thick trunk and bark properties (20-mm diameter stem) are usually presented as the main explanation for tree survival at temperatures of up to 650°C and in intense fires.[8] The tree also has potential for use in integrated planting systems as the soil water, pH, carbon, oxygen, nitrogen and phosphorus contents were found to be greater beneath *C. febrifuga*.[9] The seeds of the plant are used for fumigation while the wood is used as fuel, for building and wood works such as sculptures and domestic utensils.[10]

Ethnopharmacology

Traditional Uses

C. febrifuga, commonly known as the ordeal tree, is traditionally used in many African cultures for the treatment of different ailments and diseases. There are several documented reports on the utility of the aerial parts and roots of *C. febrifuga* by different societies in African ethnomedicine, as presented in Table 1. The leaves and the stem bark of the plant are the most utilized in the treatment or management of different ailments. The treatment of malaria is the most reported therapeutic action of the plant in African countries; Guinea,[11,12] Nigeria,[13-15] Zimbabwe,[16] Burkina Faso,[17] Ghana,[18] Mozambique,[19,20] Cote d'Ivoire, [21] Congo,[22] and Angola, [23] and the leaves and stem bark are mostly used. In Mali, Nigeria, Togo and Tanzania, the stem bark and fruits are used by traditional medicine practitioners in the treatment of upper respiratory infections such as cough, pneumonia, asthma, tuberculosis and chest pain.[14,24-27] In Cameroon and Angola, the fruits, roots or bark is used in the treatment of sterility and related ailments.[23,28,29] Other therapeutic uses of the plant include pain, fever, gastrointestinal complaints, diabetes, wound infections, sleeping sickness and epilepsy.[15,17,19, 23,27,30-35] Table 1 also reveals that all the reported medicinal benefits of the plant are from Africa, dominantly West Africa. This lends support that the plant is native to Africa.

Alternative and Complementary Medicinal Uses

An antitussive syrup, 'Improved Folk Prescription' (Balembo®) formulated with the fruits of *C. febrifuga* was produced by the Department of Traditional Medicine (DMT) in Mali.[36,37] It is known by the people and recommended for use by 76% of biomedical health workers for dry coughs.[38] It has been accepted as an essential medicine in Mali and sold in pharmacies like conventional medicines. Clinical studies done in Mali at DMT and "Dispensaire anti-tuberculeux (D.A.T)" have established the sedative effect of Balembo® syrup. For this reason, the syrup is not recommended for use by children under six months of age. Its normal dosage consists of 1 teaspoon 3 times a day.[36] Another authorized polyherbal medicine from Congo, SIROP KILMA® consisting of the stem bark of *C. febrifuga* as one of the components is also administered as a syrup for the treatment of malaria.[22] An analytical method was developed, optimized and used for the authentication and fingerprinting of KILMA® and applied for the standardization of the herbal product.[22]

Phytochemistry

Separation techniques such as thin layer chromatography (TLC), column chromatography (CC), droplet counter current chromatography (DCCC), reverse phase HPLC, Preparative Over Pressure Liquid Chromatography (Prep OPLC), Centrifugal partition chromatography (CPC) have been used to separate and isolate the chemical constituents of *C. febrifuga*. The isolated compounds were identified and/or structurally elucidated through a combination of spectroscopic techniques, including ^{1}H, ^{13}C- and 2D nuclear magnetic resonance (NMR) spectroscopy, ultraviolet-visible (UV) spectroscopy, infra-red (IR) spectroscopy and mass spectrometry.[20, 39-45] Phytochemical studies done on the fruit showed the presence of tannins, carotenoids, coumarins, mucilages, sterols, triterpenes, leucoanthocyans and saponins.[36] The major classes of compounds isolated from the plant parts of *C. febrifuga* are flavonoids, steroids, triterpenes, iridoids and sugar, occurring mostly as glycosides. These classes of compounds are typical of the Rubiaceae family [Table 2]. The isolated flavonoids, triterpenes and iridoids were mostly of the quercetin, ursane and ixoside-type skeletons, respectively. The stem bark was the most studied plant part and methanol was the more frequently used solvent of extraction. Table 2 summarises the compounds (**1**–**29**) isolated from *C. febrifuga* while Figure 3 shows the structures of the isolated compounds.

Pharmacology

Studies on *C. febrifuga* have shown that the plant possesses important pharmacological properties such as antiplasmodial, antitussive, antidiabetic, antimicrobial, gastrointestinal effects amongst others and these are summarized in Table 3. The stem bark and the leaves of the plant were investigated more than the seeds and roots. Most of the findings from these studies have corroborated the ethnomedicinal uses of the plant.

Antitussive Activity

C. febrifuga is traditionally used mainly in West Africa for the treatment of cough and respiratory disorders. Occhuito *et al*, demonstrated the antitussive activity of *C. febrifuga* stem bark in *in vivo* conditions using three experimental models; citric acid-induced cough, histamine-induced bronchoconstriction and antigen-induced bronchospasm.[24]

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Table 1: Ethnomedicinal uses of *C. febrifuga* in countries in Africa

S/No	Country	Local name	Plant part	Indications	Ref.
1.	Guinea	Mekia, Belende	leaf, stem bark	Malaria, infectious diseases	11, 12
2.	Nigeria	Kasfiya (Hausa), Ayeye (Yoruba), Ohiapele (Igala), Nambisunsun (Nupe)	leaf, stem bark	Pain, dry cough and respiratory infections, fever, dysentery, malaria, itch, skin and wound infections	13–15
3.	Zimbabwe	Chikobengwa	stem bark	Malaria	16
4.	Burkina Faso	NS	leaf	Malaria	17
5.	Ghana	Dodoyiele	leaf	Malaria	18
6.	Mozambique	NS	root, aerial parts	Fever, malaria	19, 20
7.	Congo	Mfilu, Nguala	leaf, root, stem bark	Diarrhea, convulsion Pain, malaria	22
8.	Mali	Balembo	stem bark, fruit	Cough, pneumonia, chest pain (respiratory diseases), fever, edema, diarrhea and sickness, wound healing, external and in mouth	24, 25
9.	Togo	Kesam	stem	Cough	26
10.	Tanzania	Nakapwendo	stem bark, leaf, root	Body pains, conjunctivitis, asthma, tuberculosis, constipation, abortifacient, ulcer	27
11.	Cameroon	Golombi	fruit, bark	sterility in women, ovarian cyst, threatened abortion, and syphilis	28, 29
12.	Cote d'Ivoire	NS	leaf, root	Diarrhoea, malaria, abdominal pain	21, 30
13.	Angola	Mvala	leaf, root	Sleeping sickness, epilepsy, mental disorder, headache, malaria, diabetes, cold, sexual potency, gonorrhoea	23, 34
14.	Chad	Ndeubeuh	leaf, stem bark, root	Stomach ache, fever, wound healing	35

NS = Not stated

Table 2: Compounds isolated from *C. febrifuga*

S/No	Compound	Structural class	Plant part	Extract	Ref.
1.	Vitexin	Flavonoid	leaf	70% MeOH	40
2.	Isovitexin	Flavonoid	leaf	70% MeOH	40
3.	Orientin	Flavonoid	leaf	70% MeOH	40
4.	Isoorientin	Flavonoid	leaf	70% MeOH	40
5.	Myricetin 3-galactoside	Flavonoid	leaf	70% MeOH	40
6.	Quercetin 3-rutinoside	Flavonoid	leaf	70% MeOH	40
7.	Quercetin 3-glucoside	Flavonoid	leaf	70% MeOH	40
8.	Quercetin 3-galactoside	Flavonoid	leaf	70% MeOH	40
9.	Quercetin 3-arabinoside	Flavonoid	leaf	70% MeOH	40
10.	Betulic acid	Triterpene	stem bark	DCM	40
11.	3*β*-ursa-12,20(30)-diene-27,28-dioic acid	Triterpene	stem bark, root	MeOH, DCM /Methanol (1:1)	35, 43, 44
12.	3*β*-D-glucopyranosyl-ursa-12,20(30)-diene-27,28-dioic acid	Triterpene	stem bark	MeOH	43, 44
13.	3*β*-D-3-oxoglucopyranosylursa-12,20(30)-diene-27,28-dioic acid	Triterpene	stem bark	MeOH, DCM /MeOH (1:1)	35, 43
14.	*β*-sitosterol	Steroid	stem bark	MeOH	43, 44
15.	*β*-sitosterol-3-*O*-*β*-D-glucopyranoside	Steroid	stem bark	MeOH	43, 44
16.	Spinasterol	Steroid	stem bark	EtOAc	45

17.	Hederagenin acid	Triterpene	stem bark	MeOH	43
18.	Oleanolic acid	Triterpene	stem bark, leaf	MeOH DCM / MeOH (1:1)	35, 43
19.	Ursolic acid	Triterpene	leaf	DCM / MeOH (1:1)	35
20.	3-*β*-(-α-L-rhamnopyranosyloxy)-ursa-12,20(30)-diene-27,28-dioïc acid	Triterpene	stem bark	DCM / MeOH (1:1)	35
21.	Quinovin glycoside C	Triterpene	root	DCM / MeOH (1:1)	35
22.	3*β*-*O*-*β*-D-glucopyranosyl)-28-*O*-(*β*-D-glucopyranosylester)-20(30)-ene quinovic acids	Triterpene	root	MeOH	42
23.	3*β*-(α-L-rhamnopyranosyloxy)-28-*O*-(*β*-D-glucopyranosyl)urs-12,20(30)-diene-27, 28-dioic acid	Triterpene	root bark	80% MeOH	41
24.	3-*O*-*β*-D-glucopyranosyl-2*β*,3*β*,6*β*,16α,23-pentahydroxyolean-12-en-28-oic acid 28-*O* -[α-L-rhamnopyranosyl(1→3)][*β*-D-xylopyranosyl-(1→4)][α-L-rhamnopyranosyl(1→2)]α-L-arabinopyranoside (**crossoptine A**)	Triterpene	root	MeOH	20
25.	3-*O*-[*β*-D-apiofuranosyl(1→3)] *β*-D-glucopyranosyl-2*β*,3*β*,6*β*,16α,23-pentahydroxyolean-12-en-28-oic acid 28-*O*-[*α*-L-rhamnopyranosyl(1→3)][*β*-D-xylopyranosyl-(1→4)][α-L-rhamnopyranosyl(1→2)]*α*-L-arabinopyranoside (**crossoptine B**)	Triterpene	root	MeOH	20
26.	11-methylixoside	Iridoid	root	DCM / MeOH (1:1)	35
27.	Shanzhiside methyl ester	Iridoid	stem bark, leaf	MeOH, DCM / MeOH (1:1)	35, 39, 43
28.	Shanzhiside	Iridoid	stem bark	MeOH	43
29.	D-mannitol	Sugar	leaf	DCM / MeOH (1:1)	35

The citric acid-induced experiment resulted to a dose-dependent inhibition of the number of coughs by *C. febrifuga* with the highest activity of 77.4% at 1000 mg/kg, which was higher than the control drug codeine at 76.3%. In the antigen-induced model which is widely used for testing activity against asthma, *C. febrifuga* extract displayed a protective effect against antigen-induced bronchospasm (54% inhibition) with a mean pulmonary ventilation pressure of 24.87%. The authors attributed the protective action to the effects on other pharmacological mediators involved in allergic reactions and postulated that the presence of flavonoids in *C. febrifuga* extract may partially explain its effect against antigen-induced bronchospasm. In a related study, the antitussive properties of the crude extract from *C. febrifuga* fruits was tested by the citric acid-induced method. Polysaccharides isolated from 50% aqueous ethanol were perorally administered in the dose of 50 mg/kg on guinea pigs. The results showed that there was no significant change in the number of cough efforts and specific airway resistance compared to codeine phosphate, the reference drug. Their finding contradicted the antitussive properties of Balembo®, the cough syrup from the fruits *C. febrifuga* by the Malian Department of traditional medicine which was formulated from the same composition. The authors postulated that the applied extract dose yielded a low carbohydrate content which was insufficient to elicit the pharmacological activity and attributed the low yield to the extraction procedure.[46]

Antidiabetic Activity

Diabetes mellitus is a metabolic disorder characterized by hyperglycaemia and hyperlipidemia. The leaves, root bark and stem bark of *C. febrifuga* have been studied for their antidiabetic effects. Idris and Nenge [47] tested for the antihyperglycaemic potentials of *C. febrifuga in vivo*. Thirty fasted albino rats, divided into five groups were induced with 150 mg/kg alloxan and treated with positive control (glibenclamide) and dose extracts of 500, 1000 and 1500 mg/kg *C. febrifuga* for seven days, and blood sugar levels measured each day of treatment. The blood glucose level was reduced by all the extracts but the extract of 500 mg/kg demonstrated the best activity with a more marked reduction (56.31%) compared to the positive control (28.35%). Lipid profile analysis was also done on overnight fasted rats to determine the cholesterol, triglyceride, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) of the diabetic rats. Compared to pre-treatment conditions, there was a decrease in the total cholesterol, total triglyceride and LDL (73.52% at 500 mg/kg) after treatment. The HDL however increased in all the dosed rats with the highest of 55.18% at 1000 mg/kg, but the increase was not significant as against the diabetic control group, which the authors attributed to the uninhibited actions of liplytic hormones on the peripheral fat depots due to insulin effect. Using similar experimental designs, Ojewole *et al* [48] in their study also demonstrated the hypoglycaemic and hypolipidemic of the ethanol root extracts of *C. febrifuga*.

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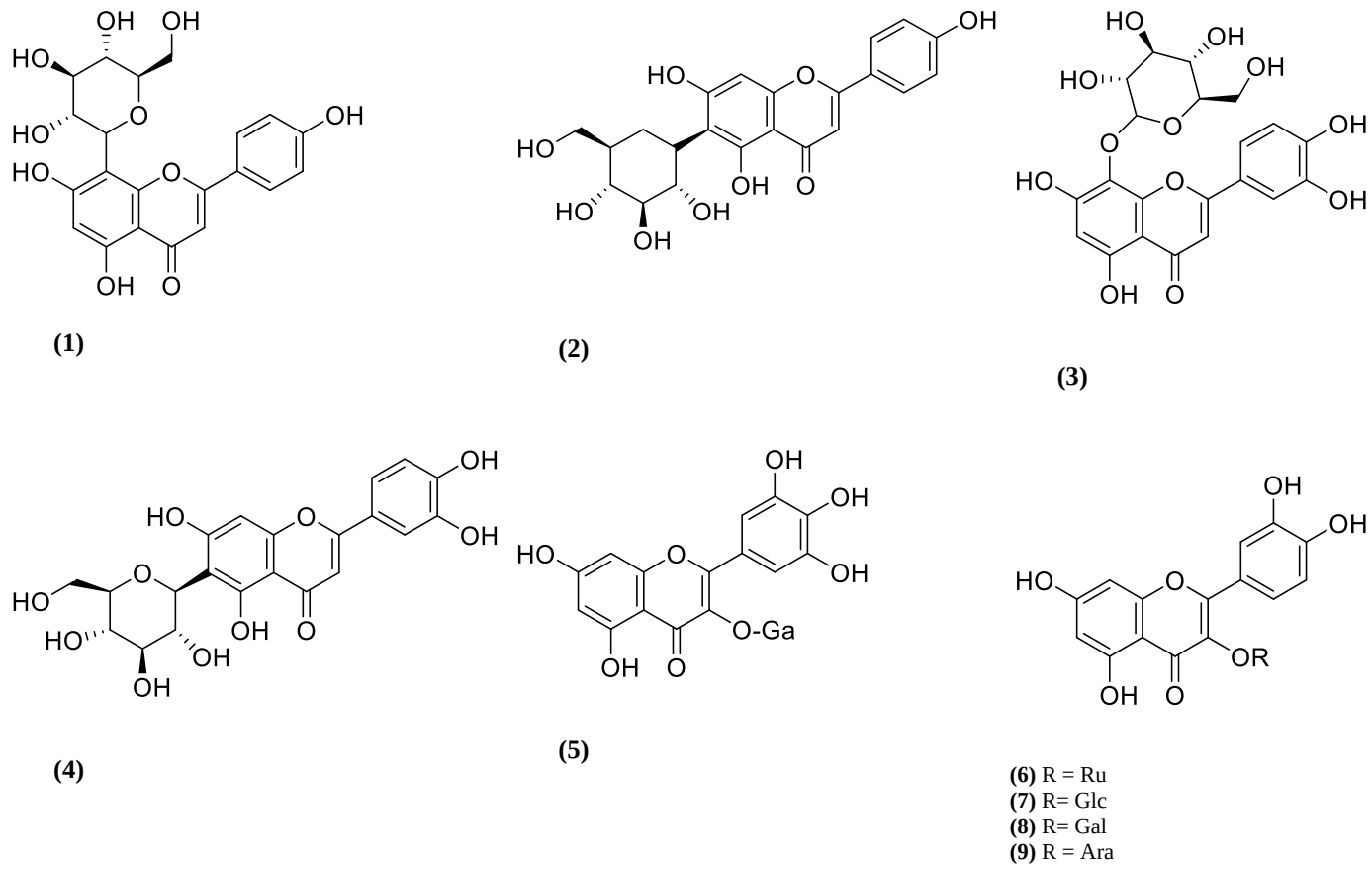

Figure 3: Flavonoids of *C. febrifuga*

Ajayi *et al*[49] also tested the methanol leaf extract of *C. febrifuga* in alloxan-induced diabetes rats and observed a significant decreased blood glucose level. In all the three different studies, the toxicity of the *C. febrifuga* extracts (LD_{50}) ranged between 1850–5000 mg/kg.

Analgesic, Anti-inflammatory and Antipyretic Activities

In many East and West African countries, *C. febrifuga* is used by the folks in the treatment of pains and fever as the specific epithet 'febrifuga' depicts. The stem bark of *C. febrifuga* was evaluated for analgesic, anti-inflammatory, and antipyretic activities in rodents. Acetic acid, oedema and brewer's yeast were employed to induce writhes, inflammation and pyrexia respectively, in the animal models. The positive controls were acetyl salicylic acid for the analgesic and anti-inflammatory studies, and dipyrone for the antipyretic studies. Three doses of the methanol extract were employed; 25 mg/kg, 50 mg/kg and 100 mg/kg. The extract caused a dose-dependent decrease in writhes and increase in pain threshold, rectal temperature, and significant attenuation in oedema for analgesia, inflammation and pyrexia, respectively.[50] The study supported the ethnomedicinal use of the plant for the relief of pain and fever.

Gastrointestinal Activity

The effect of the methanol extract of *C. febrifuga* was demonstrated against ethanol and piroxicam-induced gastrointestinal ulceration in rats.[51] The stem bark extract (25, 50 and100 mg extract/kg body weight) significantly ($P<0.05$) and dose-dependently reduced ulcer index induced by ethanol (24–92%) and piroxicam (81.81–98.60%). The authors postulated that the cytoprotective effect of the extract was due to its ability to promote secretion of bicarbonate and production of mucus. Histopathology of the rat stomach tissues from control and extract-treated groups at 25 mg/kg body weight extract showed mild inflammation characterized by infiltration of inflammatory cells, while the extract treated groups at 50 and 100 mg/kg body weight and 200 mg misoprostol/kg body weight group showed no obvious lesions. The study illustrated the safety of *C. febrifuga* in gastrointestinal tract when used as an anti-inflammatory analgesic agent that could be developed for management of painful inflammatory disorders.

Antimicrobial Activity

The antimicrobial activity of the crude methanol extract and two isolated compounds **24** and **25**, from stem bark of *C. febrifuga* were investigated *in vitro* against some bacteria and fungi. The studied microorganisms were both reference and clinical strains of *Enterobacter aerogenes, Eschericia coli, Klebsiella pneumoniae, Candida albicans, Candida parapsilosis, Cryptococcus neoformans* and *Staphylococcus aureus*. Compound **25** was most active against *K. pneumonia, S. aureus, E. coli* and *C. neoformans* with MICs between 8–64 µg/mL, the activities equal or higher than the reference compounds chloramphenicol for bacteria and nyastin for fungi. The crude extract was less active with MICs ranging between 256–1024 µg/mL.[44] Similarly, the stem bark of *C. febrifuga* was investigated, and the n-hexane, acetone and ethanol extracts were screened for their antibacterial activity against *S. aureus, K. pneumoniae* and *Pseudomonas aeruginosa*. Ampicillin was used as positive control. The acetone extract showed anti-bacterial activity at a concentration of 300 mg/mL and ethanol extract showed activity at a concentration of 100 mg/mL. No inhibition was observed on the tested microorganisms with the n-hexane extract.[52] In another study, Halilu *et al* [53] screened the methanol extract of the root bark of *C. febrifuga* against clinical isolates of some bacterial and fungal strains. The bacterial strains were *Pseudomonas aeruginosa, Staphylococcus aureus, and Eschericia coli* while the fungal strains were *Aspergillus fumigatus, Candida albicans, Aspergillus flavus* and *Aspergillus niger*. The extract showed antibacterial activity at 50 µg/mL, 100 µg/mL and 200 µg/mL on all the bacterial strains and the zones of inhibition produced ranged between 7 mm to 23 mm. The zones of inhibition produced by the

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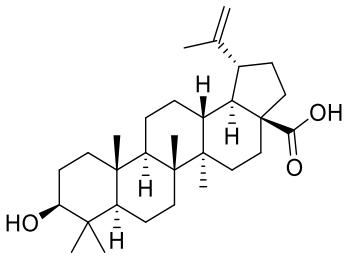
extract at 200 µg/mL compared well with the reference antibiotic ciprofloxacin.



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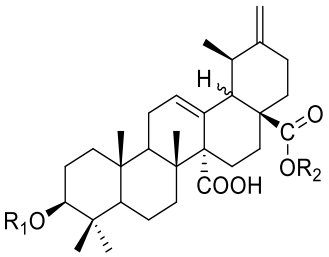
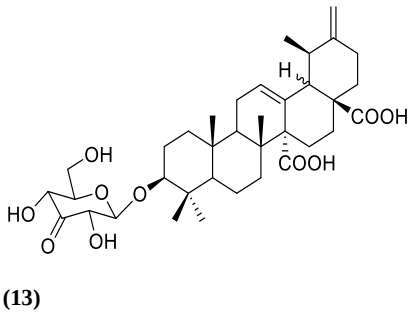


(11)* R_1= H, R_2 = H
(12)* R_1= Glc, R_2 = H
(20) R_1 = Rha, R_2 = H
(22) R_1= Glc, R_2 = Glc
(23) R_1 = Rha, R_2 = Glc



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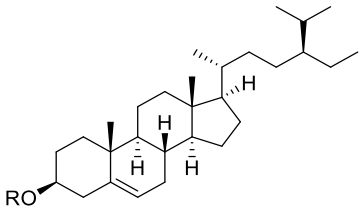
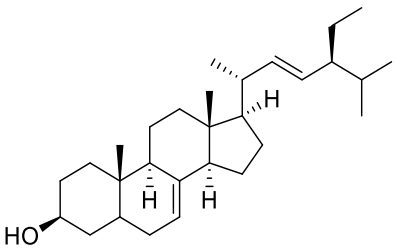


(14) R = H
(15) R= Glc



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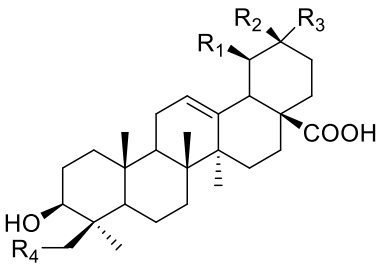
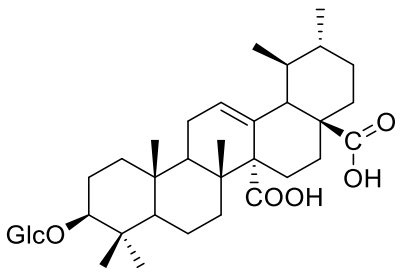


(17) R_1 = H, R_2 = R_3 = CH_3, R_4 = OH
(18) R_1 = H, R_2= R_3 =CH_3, R_4 = H
(19) R_1 = R_2 = CH_3, R_3 = H, R_4 = H



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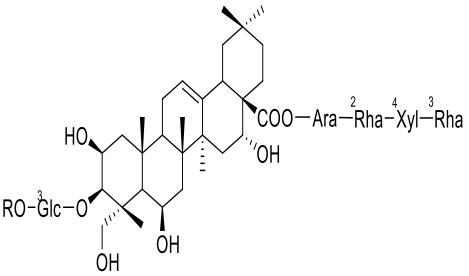
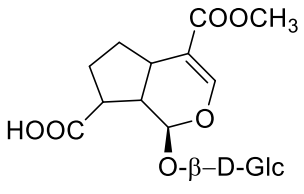
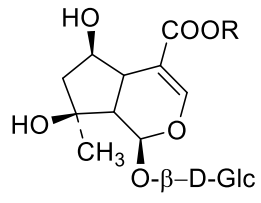


(24) R=H
(25) R= Apiose

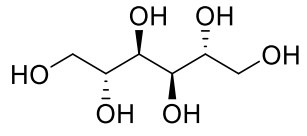
Figure 4: Steroids, triterpenes and saponins of *C. febrifuga*
*Chouna *et al* [44] isolated compounds **11** and **12** and their epimeric forms in a 3:1 ratio due to epimerisation at C-18



(26)



(27) R = CH_3
(28) R = H



(29)

Figure 5: Iridoids and sugar of *C. febrifuga*

The extract displayed better activity against the bacteria than the fungi as only one fungal strain, *A. fumigatus* was susceptible at 400 µg/mL and 500 µg/mL between the diameter range of 8–12 mm. Ouedraogo *et al*[54] also demonstrated the antibacterial properties of *C. febrifuga* (stem and leaves) where *P. aeruginosa* PAO1 was used as a reporter strain to assess the inhibition of the plant extract on pyocyanin and elastase production. The result showed that the plant exhibited antivirulence activity at 100 µg/mL with a reduction of 52% and 48%, respectively. These studies corroborate the traditional use of *C. febrifuga* for the treatment of wound and skin infections.

Antidiarrhoeal Activity

Diarrhoea is a health disorder characterized by the passage of three or more watery stools within 24 hours. In different parts of West Africa, *C. febrifuga* is administered in traditional practice for the treatment of diarrhoea. The studies by Tona *et al* [31,55] evaluated the antiamoebic and spasmolytic activities of *C. febrifuga*, along with other medicinal plants used to treat diarrhoea. A decoction of about 300 mg/mL *C. febrifuga* leaves was traditionally prepared and from the parent preparation, three extracts; phenol, saponin and alkaloid extracts were obtained. All extracts were tested for antiamoebic activity *in vitro* and antispasmolytic activity *in vivo*. The phenolic extract of *C. febrifuga* demonstrated the highest activity against *Entamoeba histolytica* with the minimum amoebic concentration of 8.5 µg/mL. For the antispasmolytic test carried out using guinea pigs, the phenol extract at 80 µg/mL also demonstrated the best activity by inhibiting the acetylcholine (Ach) and potassium chloride (KCI)-induced ileum contractions at 94.55% and 40.84%, respectively. The authors speculated that polyphenolic constituents could be considered responsible for both anti-amoebic and spasmolytic activities.[31]

Antiplasmodial Activity

In many African countries, *C. febrifuga* is reported to be used in traditional medicine for the treatment of malaria. Elufioye and Agbedahunsi[56] reported the dose-dependent, *in vivo* antiplasmodial activities of the ethanol extract of the stem bark of *C. febrifuga* in *Plasmodium berghei* infected mice. The investigation which was carried out on Swiss albino mice at a dose range of 50–400 mg/kg per day, was against early, residual (repository) and established malaria infections. Chloroquine and pyrimethamine at 5 mg/kg per day and at 1.2 mg/kg per day, respectively were used as positive controls for early and established infection tests and for residual infection test, respectively. In the early infection test, the extract gave the highest activity at 400 mg/kg with suppression at 70.97% comparable to that of the standard at 76.73% suppression. The extract was inactive in the repository test while for the established infection test, the activities of the extract at 200 and 400 mg/kg per day at 61.58% and 63.52% were comparable to that of choroquine at 66.81%. The mean survival period of the mice treated with the extract was low and this was attributed to toxicity as a result of subchronic administration of the extract. In another *in vivo* study, Salawu *et al*[50] tested the antiplasmodial efficacy of the methanol extract from the stem bark of *C. febrifuga*. Five groups of six mice each were inoculated with *P. berghei* and on the third day, doses (25, 50 and 100 mg/kg) were orally administered to three groups and chloroquine to another group at 5 mg/kg. The results showed significant and dose dependent reduction in parasite count with the highest inhibition of 84.7% at 100 mg/kg, which was higher than that of chloroquine with 76.6%. *C. febrifuga* is widely used to treat malaria in Mozambique. The *in vitro* activity of the hexane, dichloromethane, ethyl acetate and methanol extracts of the aerial parts of the plant was assessed against *Plasmodium falciparum*.[19] The dichloromethane extract showed moderate inhibition at IC_{50} = 44.4 ± 3.1 µg/mL, while the other extracts revealed no significant activity. The authors postulated that the low activity or inactivity of the extracts could be because the plant may act as an antipyretic or enhance the immune system rather than having direct antiparasitic activity. They also suggested that the plants may contain prodrugs which needed to be metabolized to bioactive molecules. Sanon *et al*. [17] also reported that an alkaloid-rich extract of *C. febrifuga* leaves exhibited antiplasmodial activity *in vitro* at 4 < IC_{50} < 10 µg/mL. The positive control was chloroquine with IC_{50} = 185 ng/ml. The studies confirmed the antimalarial potentials of the plant and its usage in traditional medicine.

Anthelminthic Activity

In their study, Kone *et al* [30] assessed the ethanol extract of *C. febrifuga* for antihelminthic activity against trematodes and nematodes and different *in vitro* assay techniques were employed against the test organisms. The roots of *C. febrifuga* demonstrated significant activity against all the organisms with Minimum Lethal Concentration (MLC) against *Echinostoma caproni* (Adults), *Schistosoma mansoni* (NTS and Adults), *Trichuris muris* (Adults) and *Heligmosomoides bakeri* (L3 larvae) *in vitro* at 160, 40, 10, 20 and 20 µg/mL, respectively hence displaying both trematocidal and nematocidal properties. On evaluation of the cytotoxicity on L6 rat skeletal myoblast cells (IC_{50}>90 µg/mL), the plant also showed no toxicity. Diehl *et al* [57] also tested the leaf extract of *C. febrifuga* against *Haemonchus contortus* and found it to be highly active with 95–100% larval mortality *in vitro*. Interestingly, the rationale for the selection of the plant for testing against the helminths arose from the traditional uses against diarrhoea, worm infections and abdominal pains.[21,30] The findings from these studies have provided more validation for its traditional uses.

Anticonvulsant Activity

Five convulsion models; Strychnine, picrotoxin, pentylenetetrazol, maximal electroshock and isonicotinic hydrazide (INH) acid-induced tests were used to evaluate of the leaf decoction of *C. febrifuga* for its anticonvulsant potentials in mice.[58] The parameters studied were the protection of the mice and the latency time of convulsion. Diazepam and clonazepam standards were used as positive control while distilled water was used as negative control. In all the induced convulsions, there was at least 50% protection of the mice at doses 80–120 mg/kg, while in the INH-induced convulsions, the latency of convulsion increased significantly as the doses of *C. febrifuga* increased. The mechanisms of action proposed by the authors included enhancement of GABA neurotransmission receptors and inactivation of voltage dependent sodium channels. The authors concluded that their study justified the continued usage of the plant for the treatment of seizures.

Table 3: Pharmacological/biological studies of crude extracts of *C. febrifuga*

S/No	Measured Activity	Plant part	Ref.
1.	Antiplasmodial	leaf, stem bark, aerial parts	17, 19, 50, 55
2.	Analgesic, ant-inflammatory, anti-pyretic	stem bark	50
3.	Gastro-protective	stem bark	51
4.	Antitrypanosomal	stem bark	59
5.	Antidiabetic	root, leaf, stem bark	47-49
6.	Antimicrobial	stem bark, root bark	44, 52, 53
7.	Antioxidant	seed, leaf	35, 60, 61
8.	Antidiarrhoeal	leaf	31, 55
9.	Anticonvulsant	leaf	58
10.	Anthelmetic	root	21, 30
11.	Toxicology	stem bark	40, 62

Anti-trypanosomal Activity

Yusuf and co-workers[59] in their study, evaluated the efficacy of *C. febrifuga* stem bark against trypanosomiasis, as claimed in ethnomedicinal practice. Using rats as the animal model, infection was established in two groups with *Trypanosoma congolense*, following the pre-latent periods for curative and prophylaxis studies. Thereafter 1500 mg/kg of the extract was administered orally for five days in succession. Analysis of the packed cell volume, red blood cell counts and differential leucocytes counts did not reveal any significant difference between the treated and untreated rats. The study did not demonstrate any prophylactic or curative activity against the parasite screened. The findings were at variance with the traditional claims of the plant being used in treating trypanosomiasis. In an ethnomedicinal survey in Angola, *C. febrifuga* was the most cited plant for the treatment of trypanosomiasis and was included in 6 different recipes and was used by specialists as well as by patients.[33] The authors attributed the wide usage of the plant to the array of disease conditions that could be treated using *C. febrifuga* and some being symptoms encountered in the course of sleeping sickness such as pain, fever, mental disorder or epilepsy, thus the rationale for using a medicinal plant known to treat such ailment. The analgesic and antipyretic activity of the plant have been previously reported.[50]

Antioxidant Activity

Oxidative stress is defined as an imbalance between stress and the available protective elements playing a crucial role in induction of diseases, such as lipid peroxidation implicated in diabetes mellitus. Antioxidants are substances that remove, prevent or delay oxidative damage to a target molecule. The seed extracts of *C. febrifuga* along with other plants, were screened for their antioxidant activities.[60] Successive soxhlet extraction was carried out to give organic, aqueous-organic and aqueous extracts. Two antioxidant procedures based on inhibition of enzymatic (lipoxygenase-catalyzed) and non-enzymatic (radical-mediated) peroxidation were employed. Quercetin was used as positive control in both assays. The results of the study showed that both assays were concentration-dependent. From the results, the best EC_{50} for radical scavenging was determined in the water extract (100°) at 34 µg/mL, and IC_{50} for enzyme inhibition in the 80% aqueous ethanol extract at 32 µg/mL. In a different but related study, Attawoidi and colleagues [61] investigated the antioxidant potentials of the leaf extract of *C. febrifuga* by evaluating the activity of two endogenous antioxidant enzymes, catalase (CAT) and superoxide dismutase (SOD). Malondialdehyde level as an indicator of lipid peroxidation was also determined. Six groups of male Albino rats were administered intraperitoneally with 10 mg/kg of the extract for three days, followed by intoxication with 0.6mL/kg of carbon tetrachloride (CCl_4). The results showed that compared to the CCl_4 control group that experienced statistically significant depletion in the levels of CAT and SOD, the levels of the enzymes in the Vitamin E control or *C. febrifuga* extract pre-treated groups were significantly boosted and also the levels of malondialdehyde in the organs were concomitantly reduced. Thus, oxidative stress generated by administration of CCl_4 was significantly prevented through pretreatment with the methanol extract of *C. febrifuga* leaf. An electrochemical method utilizing the reduction of the superoxide anion radical was employed in the antioxidant study of the extracts of the leaves, roots and stem bark of *C. febrifuga*.[35] The antioxidant activity was determined by the antioxidant index (AI_{50}) which was the concentration of extract required to consume 30% of the electrogenated radical. The stem bark demonstrated the best activity with AI_{50} of 79.50 mg/L. The findings validated the medicinal uses of the plant in the management of oxidative stress-mediated diseases.

Toxicological Studies

Investigations on the toxicity of the methanol stem bark extract of *C. febrifuga* were done by Salawu *et al*.[62] Using Swiss albino rats as the *in vivo* model, acute toxicity, sub-acute toxicity, feed and water intake, body weight change, haematology, biochemistry, relative organ weight ratio and histopathology were studied. Four groups of five rats were used for the sub-acute toxicity studies and each of the groups received saline (control), 250, 500 and 1000 mg extracts daily for 28 days. The results of the acute toxicity studies showed the oral and intraperitoneal median lethal doses to be 2828.48 mg /kg and 471.17 mg /kg, respectively. There was no mortality but reductions in feed intake, water intake and body weight were recorded for the animals after 28 days. The haemotological parameters were not significantly different from the control except the platelet count with a significant increase at all doses tested. Of the hepatic indices tested, there was a significant decrease in alanine transaminase (ALT) and aspartate transaminase (AST) at 1000 mg /kg while for the renal indices, there was a dose-dependent decrease in serum creatinine. Apart from the heart which showed a significant increase at 250 mg/kg, the relative organ weight ratio of all the organs tested were not significantly different from the control. At doses of 500 and 1000 mg/kg, histopathological changes were observed in the spleen and liver and at 1000 mg/kg for the kidney, lung and uterus. Based on the findings, the authors suggested *C. febrifuga* may be orally administered at doses lower than 500 mg extract/kg body weight and also presented cautionary advice on the use of the plants for patients with history of liver, spleen and lung diseases. In another study, the evaluation of betulinic acid (**10**) from the stem bark of *C. febrifuga* showed cytoinhibitory activities in the Co-115 human colon carcinoma cell line (LD_{50} 0.375 µg/mL) but was inactive in the human epidermal carcinoma of the nasopharynx (KB) and P-388 lymphocytic leukaemia cell lines.[40]

Conclusion

This review elaborates on the importance of this African species, *C. febrifuga* used in African alternative medicine. It presents the traditional uses, phytochemistry and various pharmacological properties including its anti-malarial, antitussive, anti-inflammatory, anti-diabetic, antimicrobial, antipyretic, anti-infective, and toxicity studies on the plant. The review also highlights the efforts made at developing the plant as a phytomedicine. Although the phytochemistry of the stem bark, leaves and roots are reported, no compound has been isolated from the fruits of the plant. Further studies need to be carried out to identify the active principles responsible for the observed pharmacological activities of *C. febrifuga*, and also to determine their mechanism of action, safety and efficacy. Quality studies on the plant are not sufficient and should also be carried out to standardize the plant towards monograph development. This will also instill confidence and gain better acceptance by the consumers of the formulated products.

Conflict of Interest

The author declares no conflict of interest.

Author's Declaration

The author hereby declares 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 her.

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