Monograph:icAcute Toxicity,Phytochemical Analysis andEthnomedicinal Study of ,PhytochemicalAnalysis,andAcuteToxicityofAqueous (Leaf and Stem BackExtracts)Extractsof three Ethnomedicinal plants from(*Macaranga hurifolia*, *Mareya micrantha*, and *Mallotus oppositifolius*) ,ThreePlantsfrom the Ivorian Pharmacopoeia on Rats.

ABSTRACT

Macaranga hurifolia (ZG02), *Mallotus oppositifolius* (ZG04), and *Mareya micrantha* (ZG08) are ethnomedicinal plants,all belonging to the Euphorbiaceae family. Which are frequently used in treating various diseases conditionsin Ivory Coast. The aim of this study is to determine the phytochemical composition and safety level of these three plants.

Phytochemical assays were conducted using tubes following standard methods. Acute toxicity was assessed according to OECD 423 guidelines. TheForty five rats were divided into fifteen 15groups of 3 three rats each.animals, (4 control groups,4controlgroups,44ZG02 groups, 4 ZG04 groups, and 3 ZG08 groups).

Results of phytochemical analysis revealed that ZG02 contains polyphenols, flavonoids, alkaloids, gallotannins, and saponins. ZG04 containsshowed the presence of polyphenols, flavonoids, catechin tannins, saponins, as well as sterols and terpenoids.While Lastly, ZG08 containspresented polyphenols, flavonoids, gallotannins, catechin tannins, saponins, as well as sterols and terpenoids.

Regarding acute toxicity,administration of the aqueous extracts for the determination of acute toxicityofZG02 and ZG04, did not produce any mortality in the rats for dosages of up to ZG02 and ZG04 did not induce any behavioral changes or mortality at doses of 300 and 2000 mg/kg of body weight. Thus, the LD50 (lethal dose for 50% of the population) of ZG02 and ZG04 is greater than 5000 mg/kg of body weight. However, the treatment of at a ZG08 atdose of 300 mg/kg of body weight,ZG08 caused a modification in the behavior of the test animals, whileand the treatment doseatof 2000 mg/kg of body weight resulted in the death of all 3 testedranimals. The LD50 of ZG08 is equal to 500 mg/kg of body weight.

In conclusion, the presence of rich active phytochemical, justifyingthe three plant extracts revealedthe presence of rich active principles, justifying their traditional uses of the plant. ZG02 and ZG04 show no oral toxicity, while ZG08 is toxic through this route.

Keywords: Ethnomedicinal, Toxicity, Aqueous Extracts, Pharmacopoeia

Formatted: Font: 10 pt

Formatted: Default

Comment [MA Kano1]: Which one is correct,5000mg/Kg or 2000mg/Kg as you mension above

Formatted: Font: 10 pt, Font color: Auto

Formatted: Font: 10 pt

Formatted: Font: (Default) Times New Roman, English (United States)

Formatted: Font: 10 pt

1. INTRODUCTION

Knowledge of tTraditional medicineknowledge in herbal medicine has been passed down through generations and it, playsing a crucial role in preserving human health [1,2,3]. Traditional healers,(custodians of this ancestral wisdom), have developed a profound understanding of the medicinal properties of plants, successfully which enableusing them tointreateing various ailmentssuccessfully, including infections [4,5,6]. Ethnobotanical research stands as an essential approach to document and preserve this traditional knowledge, thereby better understanding the richness of plant pharmacopeia [3,7,8].

It's important to note that the majority of plants listed in the NAPRALERT database are found in tropical and subtropical regions worldwide. S~~, and s~~urprisingly, biological and chemical studies of 58% of these species are poorly understood~~have not yet undergone thoroughbiological and chemical studies~~ [1,9,10]. These knowledge gaps highlight the crucial need for in-depth investigations, especially through approaches like phytochemical and pharmacological studies, including toxicity evaluation, to identify secondary metabolites and assess the safety of using these plants.

In the context of this study, the primary objective is to explore the plants most frequently cited by traditional healers for treating infections. This involves a comprehensive survey in communities where these traditional medical practices persist, shedding light on cultural specificities and local uses. The recognition of these plants by traditional medicine suggests their therapeutic potential, thereby motivating a thorough study of their chemical composition through phytochemical analysis.

Beyond the identification of chemical compounds, it's also imperative to assess the safety of using these plants. This study ~~will~~ include~~d~~an analysis of the acute toxicity of the most commonly used plants in treatement of~~ing~~ infections. It also~~,~~ provided~~ing~~ crucial information thatcan~~to~~ enlighten traditional medical practices and potentially guide modern therapeutic approaches.

Through this multidisciplinary approach, combining ethnobotanical survey, phytochemical study, and acute toxicity assessment, the aim is to better understand and valorize the rich plant heritage in service to human health.

Comment [MA Kano2]: Reconstruct this paragraph

2. MATERIAL AND METHODS

2.1 Study Area

Plant organs were harvested in Dabou, located 27 km southeast of Abidjan. These plant species were identified at the National Floristics Center of the Felix Houphouët-Boigny University Botanical Garden in Cocody (Abidjan).

2.2 Data Collection

The approach to the respondents was conducted through dialogue in French and/or the vernacular language, Adjoukrou, in Dabou.

2.2.1 Botanical and Ethnobotanical Study Parameters

The botanical study parameters encompass three (03) spectra: morphological, biological, and phytogeographical. Ethnobotanical parameters focused on plant parts used for preparation and administration of remedies, as well as the treated diseases.

Comment [MA Kano3]: Review this

2.2.2 Botanical Parameters of Recorded Plants

- **Spectrum of Morphological Types**

The determination of morphological types was established based on criteria related to size and consistency of species, as defined by Aké-Assi [11]. In this study, they were simplified into five major groups: trees, shrubs, subshrubs, herbs, and vines.

- **Spectrum of Biological Types**

Biological type indicates the adaptive behavior of the species and provides information about the vegetation formation, its origin, and transformations. It was determined following Raunkiaer's system (1934) adapted to tropical vegetation [11,12]. These included Phanerophytes (mega, meso, micro, and nano), Chamaephytes, Hemicryptophytes, Geophytes, and Therophytes.

- Phytogeographical Spectrum

Phytogeography studies the distribution of plant species across the globe [13]. The phytogeographical characterization of species was done using Aké-Assi's distribution types (2001; 2002). These encompassed species from the Guineo-Congolian Region (GC); species from savanna, open forest, or steppe of the Soudano-Zambezian Region (SZ); species present in both the Guineo-Congolian and Soudano-Zambezian Regions (GC-SZ); introduced or cultivated species (I).
Ethnobotanical study parameters focused on: plant parts used (leaves, stem bark, roots, stem, seeds, flowers, whole plant) for preparing and administering remedies, and the treated diseases.

2.3 Acute Toxicity

The plant material consisted of stem bark powder from *Macaranga hurifolia* and leaves from *Mareya micranthaand Mallotus oppositifolius.*

2.3.1 Preparation of Extract

The collected leaves and stem bark were dried under controlled conditions at 18°C for two weeks before being pulverized into a powder. One hundred (100) grams of powder from each sample were grinded anddissolved~~extracted~~ in one liter of distilled water~~ by grinding in a Moulinex blender for 10 to 15 minutes~~. The resulting homogenates were first squeezed through a clean white cloth, then successively filtered using hydrophilic cotton and Whatman filter paper no.3. After filtration, the obtained filtrates were dried by evaporation in a venticell-type oven at 50°C. The resulting powders constituted the total aqueous extracts, labeled as ZG02 (*Macaranga hurifolia*), ZG04 (*Mallotus oppositifolius*), and ZG08 *(Mareyamicrantha)*. They were then stored in sterile glass jars and kept refrigerated at 4°C until use.

Comment [MA Kano4]: Review this statement

2.3.2 Animal Material

The animal material consisted of 45 virgin nulliparous female rats (*Rattus norvegicus* strain Wistar), aged 45 to 50 days and weighing between 115 and 130 grams. These animals were selected according to the method described in OECD Guideline 423 (Organisation for Economic Co-operation and Development) in paragraphs 11 and 12 [14]. The rats were housed in a room with a constant temperature of 24±2°C, a 12-hour natural light and 12-hour dark photoperiod. Humidity levels were maintained at 50 to 55%, and the animals had free access to water and food (pellets, 15% protein, 4% fat) provided by Faci-Abidjan. All these measures complied with OECD recommendations in paragraph 13 [14].

Comment [MA Kano5]: Review this statement

2.3.3 Acute Toxicity Method

Acute toxicity was conducted following OECD Guideline 423 [14]. According to paragraph 9, the extracts were tested sequentially, using three rats per batch at each stage for each plant extract.

Comment [MA Kano6]: Review this statement

Due to insufficient precise information on the toxicity of the studied plant extracts at a dose of 2000 mg/kg body weight, an initial dose of 300 mg/kg body weight was used in line with paragraph 19 of the guideline [14].
In total, 45 rats were divided into 15 groups of 3 animals each (4 control groups, 4 ZG02 groups, 4 ZG04 groups, and 3 ZG08 groups)~~, and fastedovernight before extract administration~~. After overnightfasting, the animals were individually marked for identification and weighed. Then a single dose of 300 mg/kg body weight of each extract was administered to a group using a gastric tube as follows:

Group 1 (control: 3 rats): distilled water

Group 2 (treated: 3 rats): ZG02 at 300 mg/kg body weight

Group 3 (treated: 3 rats): ZG04 at 300 mg/kg body weight

Group 4 (treated: 3 rats): ZG08 at 300 mg/kg body weight

Following extract administration, the animals were again deprived of food for 3 to 4 hours. Moreover, the animals were individually observed at least once during the first 30 minutes and regularly for the first 24 hours. Special attention was given for the initial 4 hours and daily for 14 days after extract administration. All animals were observed at least twice daily to potentially record any signs of pathology or behavioral changes.

Comment [MA Kano7]: Reconstruct this

Observations included changes in skin, fur, eyes, and mucous membranes, as well as the respiratory system, circulatory system, autonomic and central nervous systems, somatomotor activity, and behavior. Particular attention was paid to observing various manifestations such as tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma.
The absence or presence of mortality related to an extract in a group receiving the dose of 300 mg/kg body weight determined the next step, namely:
Administering the same dose (300 mg/kg body weight) of the extract to three additional animals (repeat of the previous test) if there were no more than 1 death in the batch.

Administering the immediately higher dose (2000 mg/kg body weight) to three additional animals if there were 0 or 1 death in the previous tests (test and repeat of the test) with the dose of 300 mg/kg body weight (this latter test of 2000 mg/kg body weight is repeated if there were no more than 1 death in a batch).

Comment [MA Kano8]: Review and reconstruct it

2.4 Phytochemical Screening Methods

It involves characterizing or identifying the main chemical groups of therapeutic interest in a plant using a suitable extraction method. The tri-phytochemical assays were performed in test tubes.

The search for alkaloids was conducted using the Dragendorff technique (reactive with potassium iodobismuthate) and the Bouchardat technique (iodine-iodide reagent) [15,16,17]. Polyphenols were detected using the ferric chloride ($FeCl_3$) reaction [18]. Flavonoids were identified through the "cyanidine" reaction [19]. Saponins were identified based on their physical property: the formation of persistent foam upon agitation [20]. Catechol tannins were detected using the Stiasny reagent: 30% formaldehyde, concentrated HCl in a ratio of 1:0.5 [21]. Gallotannins were identified through the reaction with 2% sodium acetate and ferric chloride [22]. Coumarins were detected using a methanolic potassium hydroxide (KOH) solution at 10% (v/v) neutralized with 10% (v/v) hydrochloric acid (HCl). Sterols and polyterpenes were identified using the Liebermann reaction [16,17].

Comment [MA Kano9]: You have to briefly explain each method

e.g.Saponin Determination
The method of Obadoni and Ochuko (2001) was used. Out of the grinded samples 10g was weighed for each and put into a conical flask and 100ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4hours with continuous stirring at about 55oC. The mixture was filtered and the residue re-extracted with another 200 ml, 200 % ethanol. The combined extracts were reduced to 40 ml over water bath at about 90 oC. The
concentrate was transferred into a 250 ml separator funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 ml of n – butanol was added. The combined n-butanol extracts were washed
twice with 10 ml of 5 % aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponins content was calculated as percentage.

3. RESULTS AND DISCUSSION

The significance of traditional plants in treating infections is widely acknowledged. This study was initiated specifically in Dabou, within the Grand Ponts region, to catalog plants used in infection treatments [23,24]. The research identified three plant species commonly employed in treating various infections. This supports the already-established diversity of Ivorian medicinal flora mentioned in previous works [9,25].

Three plants were selected based on their frequency of citation and usage. These are *Mallotus oppositifolius* (zg04), *Mareya micrantha* (zg08), and *Macaranga hurifolia* (zg02). These results led to the study of acute toxicity. The results of the botanical and ethnobotanical parameters are recorded in appendix 1.

M. Oppositifolius (geisel.) Müll.-arg is a shrub approximately 6 meters tall. The young shoots are covered with star-shaped hairs, while older branches are nearly glabrous. The leaves are simple and opposite (figure 1). Each pair has a long petiole and a slightly thickened short petiole at both ends. The stipules are very small and quickly fall off. The blade is broadly oval of unequal size on each pair with a weakly rounded or truncated base. The margin is almost entire, more or less deeply toothed or lobed with three veins starting from the base, adorned with scattered star-shaped hairs. The inflorescence is a terminal or axillary cluster. The flowers are unisexual, fragrant without petals, with numerous stamens. The fruits consist of three lobes with smooth, shiny grains of a brown-grayish color [23].
M. oppositifolius is a shrub that colonizes the understory of secondary forests. It also grows at the forest edge and in associated bushes or thickets, as well as along rivers, from sea level up to 1650 meters altitude. This plant is widely distributed. It is found from Senegal to Ethiopia, southwards to Angola and Mozambique, as well as in Madagascar [26].

Comment [MA Kano10]: This should come after presenting the result.

You have to start presenting your result (Tables), followed by discuss

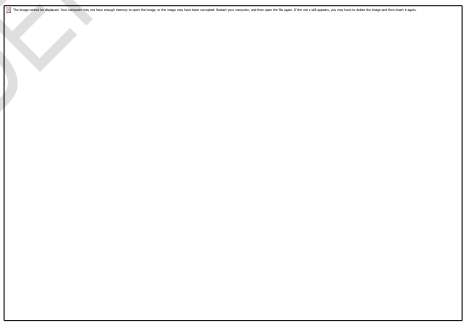

Figure 1: leafy branches with inflorescences *Mallotus oppositifolius* (Geisel) Müll.Arg (Euphorbiaceae) [27].

M. oppositifolius is used to address multiple pathologies. In Nigeria and Ghana, leaf decoction is used for treating convulsions, epilepsy, eye infections, headaches, and ringworms [28,29]. Additionally, root decoction is employed against anemia, pneumonia, paralysis, asthma attacks, and chewed for oral hygiene [30]. In East Africa, as per Chhabra et al. [31], root decoction is taken as an aphrodisiac. A steam bath of this preparation is suggested for treating headaches and mental illnesses. In the Democratic Republic of Congo, crushed leaves infused in saltwater are imbibed to counteract

snakebite venom. Similarly, crushed leaves macerated in palm wine are recommended for managing urinary infections, venereal diseases, malaria, chickenpox, and female infertility [32]. In Ivory Coast, calcined roots are used to treat Buruli ulcers, while leaves are recommended for chronic wounds, diarrhea, and urinary infections [33].

Mareya micrantha (Benth.) Müll. Arg is a monoecious shrub reaching 8 to 12 meters in height, with branches bearing short hairs. The leaves are simple and alternate. The small stipules are triangular, falling off rapidly; the petiole is long; the blade is oval, with a cuneate base, shortly acuminate apex, slightly dentate in the upper part. The inflorescence is a slender axillary raceme reaching 25 to 40 cm in length, with male flowers in clusters in the upper part and solitary female flowers or accompanied by several male flowers in the lower part (Figure 2). The flowers are unisexual; petals are absent; the calyx opens in 3–4 lobes, approximately 1.2 mm long, obtuse, green, with 10–20 (–24) stamens, longer than the calyx lobes, free; female flowers almost sessile, with 3–5 sepals, about 1 mm long, imbricate, greenish, with a superior, ovoid ovary, with short hairs. Fruit: a 3-lobed capsule, 3–4 mm in diameter, slightly depressed above, with short hairs, light brown to reddish, containing 3 seeds. Seeds are ovoid, about 2 mm in diameter, smooth, brownish [34].
M. micrantha is found from Guinea to Cameroon and the Democratic Republic of Congo.
The leaves of *M. micrantha*, when crushed and macerated in water, yield a filtrate. One glass of this liquid may be used for purging, but an excessive dose can be fatal. Both the leaves and fruits, highly bitter and toxic, induce a severe purging effect when consumed. The leaf decoction or juice is known for its strong purgative and abortive properties. Even when diluted, fresh leaf decoction is never given to pregnant women, children, or the elderly. It is primarily used to treat conditions such as tapeworm infections and gonorrhea. However, a decoction of dried leaves is given to children as a vermifuge [35]. Burnt leaves mixed with clay are applied for scabies and measles. The decoction of leaves or fermented leaves with rum and coconut is used for coughs. Externally, leaf paste is applied to wounds and ulcers, especially those caused by Guinea worms. Root powder is applied to snakebites and venomous animal stings.

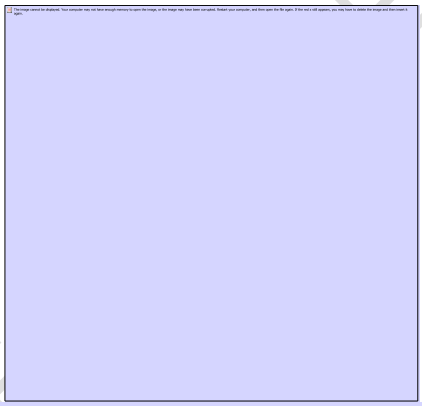

Figure 2: Leaves and leafy branch with inflorescences of *M. micrantha* (Benth.) Müll. Arg. (Euphorbiaceae). (Yapo, 2014; Zirihi board, 2006)

Macaranga hurifolia Beille is a spiny shrub or tree reaching 12m-15m in height. It is a dioecious species with stilt roots. The fruit is a capsule containing a single seed, approximately 2 mm in diameter [36]. The wood is white, of moderate texture, and easily worked, suitable for many of the same purposes as soft pine (Figure 3).
M. hurifolia ranges from secondary jungle, from Sierra Leone to Cameroon.
The stem bark of *M. hurifolia* is used externally for edema. Leaf maceration is employed for treating coughs. An aqueous maceration of leafy branches, often with *Baphia nitida*, acts as a purgative for various gastrointestinal ailments.

Comment [MA Kano11]: This should be after result and discusion

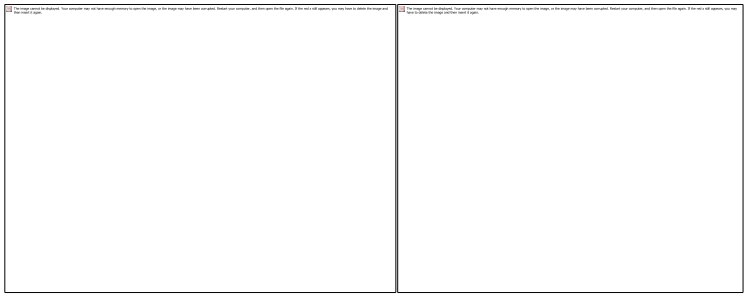

Figure 3: Bark incision of stem and leafy branches with inflorescences of *Macaranga hurifolia* Beille.

The results of the phytochemical studies are recorded in table 1. The phytochemical study of the aqueous extract of *Macarangahurifolia* (zg02) has shown that, the phytochemical composition of the plant are ~~identified the presence of~~ polyphenols, flavonoids, alkaloids, gallotannins, and saponins. These findings corroborate Sylla *et al*. conclusions [37], ~~who also highlighted these compounds in their studies on the same plant~~. Similarly, the aqueous extract of *Mallotus oppositifolius* (zg04) revealed the presence of polyphenols, flavonoids, catechin tannins, saponins, ~~and~~ sterols and ~~terpenoids~~terpenoids. It is also, ~~align~~ing with the discoveries of Pissang et al. ~~in their investigations of this plant~~ [38]. The study of the aqueous extract of mareya micrantha (zg08) showed the presence of polyphenols, flavonoids, gallotannins, catechin tannins, saponins, and sterols and terpenoids, except for alkaloids absent in zg08, confirming Ladoh-Yemeda et alearlier findings on the same plant [39].

The richness in active chemical compounds in these plants might explain their traditional use in treating various conditions. For instance, *Macaranga hurifolia* is used in treating cough and diabetes, according to Tra-Bi work [40]. Ethnobotanical studies in the Transua Department, Zanzan District (Côte d'Ivoire) by Béné et al. [25] indicate that *Mallotus oppositifolius* is traditionally used for external bleeding, while *Mareya micrantha* is recommended for hemorrhoids, hypertension, and bloating treatment.

Furthermore, the identified compounds exhibit beneficial pharmacological activities for mammalian body functions. Polyphenols are known for their cardiovascular properties and their role against degenerative diseases. Flavonoids are associated with hepatoprotective, anti-inflammatory, and antioxidant activities. Alkaloids, aside from their estrogenic effects, possess antioxidant, anti-inflammatory, anticonvulsant, and analgesic properties. Tannins demonstrate antibacterial, antifungal, and antiviral activities. Saponins have estrogenic, androgenic, and aphrodisiac effects, while sterols and terpenoids are recognized for their anti-inflammatory activity.

Comment [MA Kano12]: Reconstruct this

Comment [MA Kano13]: The discussion should be after table

You to present tables first

Table 1: Chemical composition of *Macaranga hurifolia* stem bark (ZG02), *Mallotus oppositifolius* leaves (ZG04), and *Mareya micrantha* leaves (ZG08)

Plants extracts / **Chemical compound**		ZG02 ETA	ZG04 ETA	ZG08 ETA
Polyphénols		+++	++	+++
Flavonoïdes		++	+	++
Alcaloïdes		+	-	-
Tanins	galliques	+	-	+
	catéchiques	-	++	+
Saponosides		++	++	+++
Stérols et terpénoïdes		-	+	++
Coumarines		-	-	-

-: Absence +: Presence ++: High presence +++: Very high presence; ZG02: *Macaranga hurifolia*, ZG04: *Mallotus oppositifolius*, ZG08: *Mareya micrantha*; TAE: Total Aqueous Extract

Regarding acute toxicity,administration of the aqueous extracts for the determination of acute toxicity of ZG02 and ZG04, did not produce any mortality in the rats for dosages of up to 2000 mg/kg~~The administration of ZG02 and ZG04 to the rats did not induce any behavioral changes and did not result in any deaths at doses of 300 and 2000 mg/kg body weight~~ (Table 2 and 3). However, treatment of ZG08 at ~~a dose of~~ 300 mg/kg body weight induced drowsiness, reduced mobility, and respiratory rate (Table 2). The dosage~~e~~ of 2000 mg/kg body weight of this latter extract led to loss of appetite, convulsions, reduced mobility and respiratory rate, drowsiness progressing to lethargy, slowing of heart rate resulting in the death of all 3 test animals (Table 2 and 3).

Comment [MA Kano14]: This should be after (below)the table

Table 2: Observed Parameters after Oral Administration of 300 mg/kg BW of ZG02, ZG04, and ZG08.

Toxicity Signs	Test with the dose of 300 mg/kg BW			
	Control	**ZG02**	**ZG04**	**ZG08**
Loss of appetite	-	-	-	-
Skin and fur	-	-	-	-
Eyes	-	-	-	-
Mucous membrane	-	-	-	-
Salivation	-	-	-	-
Lethargy	-	-	-	-
Sleep	-	-	-	+
Coma Mobility	-	-	-	-
Convulsion	-	-	-	-
Diarrhea	-	-	-	-
Mobility	-	-	-	+
Respiratory rate	-	-	-	+
Heart rate	-	-	-	-
Moribund	-	-	-	-
Mortality	-	-	-	-

(-)= Absence of sign**; (+)** = Presence of sign**;** ZG02: *M. hurifolia*, ZG04*: M. oppositifolius*, ZG08: *M. micrantha*

Table 3: Observed Parameters after Oral Administration of 2000 mg/kg BW of ZG02, ZG04, and ZG08.

Toxicity Signs	Test with the dose of 2000 mg/kg BW			
	Control	**ZG02**	**ZG04**	**ZG08**
Loss of appetite	-	-	-	+
Skin and fur	-	-	-	-
Eyes	-	-	-	-
Mucous membrane	-	-	-	-
Salivation	-	-	-	-
Lethargy	-	-	-	+
Sleep	-	-	-	+
Coma Mobility	-	-	-	-
Convulsion	-	-	-	+
Diarrhea	-	-	-	-
Mobility	-	-	-	+
Respiratory rate	-	-	-	+
Heart rate	-	-	-	+
Moribund	-	-	-	-
Mortality	-	-	-	+

(-) = Absence of sign; **(+)** = Presence of sign; ZG02: *M. hurifolia*, ZG04*: M. oppositifolius*, ZG08: *M. micrantha*

The toxicological study, following OECD 423 guidelines, revealed no signs of toxicity or mortality after the administration of the limit dose (2000 mg/kg BW) of the aqueous extracts of *Macaranga hurifolia* (ZG02) and *Mallotus oppositifolius* (ZG04). According to the OECD's Globally Harmonized System (GHS) (2001), these extracts fall under category 5, which means they are not classified as toxic. The LD50 values for ZG02 and ZG04 are greater than or equal to 5000 mg/kg BW, hence classified as non-toxic, findings consistent with Affy et al. [41] regarding the acute toxicity of the aqueous extract of *Amaranthus viridis* (Amaranthaceae).

However, the administration of the aqueous extract of Mareya micrantha (ZG08) induced adverse effects. At the dosagee of 300 mg/kg BW, signs like drowsiness, reduced mobility, and decreased respiratory rate were observed. At the dosagee of 2000 mg/kg BW, more severe effects such as loss of appetite, drowsiness, lethargy, convulsions, reduced mobility, decreased respiratory and heart rates were observed, leading to the death of the three test animrats. These results suggested that the active compounds present in ZG08 can cause severe dysfunctions beyond their expected therapeutic effects. For instance, polyphenols are known to cause gastrointestinal burns, cyanosis, hypoxia, and convulsions when ingested or inhaled. Similarly, sterols are associated with surfactant and hemolytic properties, and some sterols, like tetracyclic triterpenes, are known for their necrotizing and cytotoxic properties in rodents. According to the OECD's Globally Harmonized System (GHS) (2001), this extract is classified under category 4, with an estimated LD50 of 500 mg/kg BW, placing it at the upper limit of toxicity according to Diezi (1989).

4. CONCLUSION

The phytochemical study of the aqueous extracts of *Macaranga hurifolia, Mareya micrantha*, and *Mallotusoppositifolius* highlighted that, there areepresence of abundant active compounds, thus justifying their traditional use. The results of the acute toxicity study revealed that,*M. hurifolia* and *M. oppositifolius*haveshow an LD50 greater than or equal to 5000 mg/kg body weight, classifying them as non-toxic (ZG02 and ZG04). However, *Mareya micrantha* displays an LD50 of 500 mg/kg body weight, indicating toxicity. Nonetheless, to gain a more comprehensive understanding of safety for use, it's imperative to continue this study through investigations on sub-acute and chronic toxicity.

REFERENCES

1. Awoyemi OK, Ewa EE, Abdulkarim IA, Aduloju AR. ethnopharmacology and drug. Academic research international. 2012; 2: 50- 57.

2. Mambé FT,Voukeng IK, Beng VP. Antibacterial activities of methanol extracts from *Alchornea cordifolia* and four other Cameroonian plants against MDR phenotypes. Journal of Taib University, Medical Sciences. 2016; 11(2): 121-127.

3. Dieye PI, Sarr SO, Ndiaye N. Status of research for antimicrobial target molecules from plants in Africa. Afrique SCIENCE. 2020; 16(1), 348-374.

4. Koudouvo K, Esseh K, Denou A, Aziati T, Ajavon C, Afanyibo YG, Agbonon A, Sanogo R, Dougnon J, Aklikokou K, Aguiyi JC, Diallo D, Mensah GA, Gbeassor M. Ethnopharmacological study of antimalarial recipes in Togo for a formulation of phytomédecine for malarial taking care. Bulletin de la Recherche Agronomique du Bénin (BRAB) Numéro. 2016; 79: 1025-2355.

5. Assouma AF, Koudouvo K, Diatta W, Vidzro MK, Guelly AK, Dougnon J, Agbonon A, Tozo K, Gbeassor M. Ethnobotanical Survey on the Traditional Management of Female Infertility in the Health Region. European Scientific Journal, 2018 ; 2 :12-26.

6. Esseh K, Afanyibo YG, Ahama-Esseh KYS, Idoh K, Koudouvo K, Agbonon A, Gbeassor M. Screening Phytochimique, Étude Toxicologique, Évaluation des Activités Antiplasmodiale et Antiradicalaire de la Tige Feuillée de Senna occidentalis Linn (Fabaceae). European Scientific Journal, February 2019 edition; 411(15) : 411-433.

7. Leonti M, Stafford GI, Dal CM, Cabras S, Castellanos ME, Casu L, Weckerle CS. Reverse ethnopharmacology and drug discovery. Journal of Ethnopharmacology, 2017 ; 198, 417-431.

8. Aboudou AD, Koffi k. Ethnobotanical Investigation of Medicinal Plants Used in the Traditional Management of Infectious Diseases in the Lomé/Commune Health Region of Togo. European Scientific Journal, 2021 ; 17(21): 46-65

9. Ambe AS, Ouattara D, Tiebre MS, Vroh BTA, Zirihi GN, N'guessan KE. Diversity of medicinal plants used in the traditional treatment of diarrhea in the markets of Abidjan (Ivory Coast). Journal of Animal & Plant Sciences. 2015; 26(2), 4081-4096.

10. Agody M, Bakoma B, Batawila K, Wala K, Dourma M, Pereki H, Dimobe K, Bassene H, Akpagana K. Contribution to the census of medicinal plants in Togo: Case of the Maritime Region. European Scientific Journal. 2019 ; 15(24) 329-345.

11. Aké-Assi L. Flora of Ivory Coast: systematic catalog, biogeography and ecology. Geneva. Boissiera. 2001; 57:1-396. French.

12 Aké-Assi L. Flora of Ivory Coast: systematic catalog, biogeography and ecology. Geneva. Boissiera. 2002; 58:1-401. French.

13. Zougrou NE. Evaluation of the pharmacological effects of an aqueous extract of Cnestis ferruginea Vahl ex Dl. (Connaraceae) on the reproductive system of male and female rats. Single Doctoral thesis, Felix Houphouët University – Boigny, Abidjan (Ivory Coast). 2017, 246p. French.

14. OECD. OECD Guideline for Testing of Chemicals: Acute Oral Toxicity – Acute Toxicity Class Method. OECD. 423, 2002; 14p. French.

15. Diallo AM. Study of medicinal plants from niafunke (Timbuktu region) phytochemistry and pharmacology of Maerua crassifolia Forsk. (Capparidaceae). Doctoral thesis from the University of Bamako, 2005; 125p. French.

16. Muanda NF. - Identification of polypenols, evaluation of their antioxidant activity and study of their biological properties. Doctoral thesis from Paul Verlaine University, Metz, France, 2010; 294 pp. French.

17. Attou A. Contribution to the phytochemical study and biological activities of extracts of the plant Rutachalepensis (Fidjel) from the Ain Témouchent Region. Master's Thesis in Biology Tlemcen-Abou Bekr Belkaid University, Algeria, 119p. French.

18. Esseh K, Afanyibo YG, Ahama-Esseh KYS, Idoh K, Koudouvo K, Agbonon A, Gbeassor M. Phytochemical Screening, Toxicological Study, Evaluation of Antiplasmodial and Antiradical Activities of the Leafy Stem of Senna occidentalis Linn (Fabaceae). European Scientific Journal. 2019; 411(15): 411-433.

19. Lhuillier A. Contribution to the phytochemical study of four Malagasy plants: Agauriasalicifolia Hook. F ex Oliver, Agauria polyphylla baker (Ericaceae), Tambourissa trichophylla Baker (Monimiaceae) and Embelia concinna Baker (Myrsinaceae). France: National Polytechnic Institute of Toulouse (France), 2007; 214 pp. French.

20. Fofana S, 2004.- Biochemical exploration of the immunogenic power of three plants in Ivory Coast: Alstonia boonei (apocynaceae), Mitragyna ciliata (rubiaceae) and Terminalia Catappa (combretaceae). Doctoral thesis in Pharmacy, University of Cocody-Abidjan, Ivory Coast, 123 p. English

21. Tra Bi FH, Irié GM, N'Gaman KCC, Mohou CHB. Studies of some therapeutic plants used in the treatment of high blood pressure and diabetes: two emerging diseases in Ivory Coast. *Sciences & Natures*. 2005 ; 5 (1): 39-48.

22. Mogode DJ. Phytochemical and pharmacological study of Cassia nigricans Vahl (Caesalpiniaceae) used in the treatment of dermatoses in Chad. Doctoral thesis in Pharmacy, University of Bamako, Mali, 234 p. French.

23. Yapo CVY, N'Dri GK, Kiyinlma C, Djeneb C, Guédé NZ. Botanical study, evaluation of antifungal activity on in vitro growth of C. albicans and toxicity on HFF cells of mallotus opposifolius. Journal of Animal & Plant Sciences. 2016; 28(1):4330-4339.

24. Yapo YCV. Antimicrobial medicinal plants used in the Grand Ponts region (Ivory Coast): Ethnobotanical study and evaluation of the antibacterial, antifungal and toxic activities of the most used taxa. National Doctoral Thesis, Felix Houphouët-Boigny University, (Ivory Coast), 2018; 178p. French

25. Béné K, Camara D, Fofié NBY, Kanga Y, Yapi AB, Yapo YC, Ambé SA, Zihiri GN. Ethnobotanical study of medicinal plants used in the Transua department, Zanzan District (Ivory Coast). Journal of Animal & Plant Sciences, 2016; 27 (2): 4230-4250.26. Gnagne AS, Camara D, Fofié NBY, Béné K, Zirihi GN, 2017. Étude ethnobotanique des plantes médicinales utilisées dans le traitement du diabète dans le Département de Zouénoula (Côte d'Ivoire), Journal of Applied Biosciences 113: 11257-11266.

27. Yapo YCV. Etude ethnobotanique des plantes médicinales antipaludiques vendues sur les marchés de la commune de Cocody et d'Abobo (Abidjan, Côte d'Ivoire). Mémoire de Master II de l'Université de FHB, UFR Biosciences. 2014 ; 50 p. French

28. Ngoule C, Ngene J, Kidick P, Ndjib R, Dibong S, Mpondo ME. Inventaire et caractérisation floristiques des plantes médicinales à huiles essentielles des marchés de Douala Est (Cameroun). International. Journal of Biological and. Chemical Sciences. 2015 ; 9 (2) : 847-889.

29. N'Guessan K, Tiébre MS, Aké-Assi E, Zirihi GN, Ethnobotanical study of plants used to treat arterial hypertension, in traditional medicine, by Abbey and Krobou populations of Agboville (Côte d'Ivoire). European Journal of Scientific Research, 2009; 35 (1) : 85- 98.

30. Klotoé JR, Dougnon TV, Koudouvo K, Atègbo JM, Loko F, Akoègninou A, Aklikokou K, Dramane K, Gbeassor M. Ethnopharmacological survey on antihemorrhagic medicinal plants in South of Benin. European Journal of Medicinal Plants, 2013; 3(1) : 40-51.

31. Chhabra SC, Mahunnah RLA, Mshiu EN. Plants used in traditional medicine in eastern Tanzania. 3. Angiosperms (Euphorbiaceae to Menispermaceae). Journal of Ethnopharmacology 1990; 28 : 255–283.

32. Yapi AB. Inventaire des plantes médicinales de la famille des Asteraceae des marchés de la commune d'Abobo (Abidjan, Côte d'Ivoire). Mémoire de Master II de botanique, UFR Biosciences, Université Félix Houphouët-Boigny, 2013 ;50 p. French.

33. Koudouvo K, Denou A, Asseh K, Sanogo R, Essien K, Diallo D, Kokou K, Tozo SK, Aklikokou K, Aguiyi JC, Gbéassor C. Ethnobotanical Survey of Endangered Antimalarial and Analgesic Plants of Togo for the Safeguard of the Medicinal Biodiversity. Journal of Agriculture and Ecology Research International. 2017; 12(2):1-9

34. Koulibaly A, Monian M, Ackah JAAB, Koné MW, Traore K. Étude ethnobotanique des plantes médicinales : cas des affections les plus fréquentes d'une région agricole Daloa (Centre Ouest, Côte d'Ivoire). Journal of Animal &Plant Sciences, 2017 ; 31 (2) : 5021-5032.

35. Lakouéténé DPB, Ndolngar G, Berké B, Moyen J-M, Kosh Komba E, Zinga I, Silla S, Millogo-Rasolodimby J, Vincendeau P, Syssa-Magalé J-L, Nacoulma-Ouedraogo OG, Laganier R, Badoc A. Chèze C. Enquête ethnobotanique des plantes utilisées dans le traitement du paludisme. European Scientific Journal August. 2019 ; 15:(24)1857-7881.

36. Mpondo ME, Ngene JP, Som ML, Etame LG, Boumsong NPC, Yinyang J, Dibong SD. Connaissances et usages traditionnels des plantes médicinales du département du haut Nyong. Journal of applied Biosciences 2017; 113 : 11229-11245.

37. Sylla Y, Silue DK, Ouattara K, Kone MW. Ethnobotanical study of plants used against malaria. Bulletin of the Bordeaux Pharmacy Society. 2018; 148:123-138. French

38. Pissang P, Agban A, Yao PH, Tchacondo T, Sadji AY, Effoe S, Batawila K. In Vitro Evaluation of the Antimicrobial Activity of Cassia alata Linn Extracts. (Fabaceae). European Scientific Journal, 2016; 12(21).

39. Ladoh-Yemeda CF, Vandi T, Dibong SD, Mpondo EM, Wansi JD, Betti JL, Choula F, Ndongo D, Eyango MT. Ethnobotanical study of medicinal plants sold in the markets of the city of Douala, Cameroon. Journal of Applied Biosciences, 2016; 99(1), 9450-9466.

40. Tra Bi FH, Irié GM, N'Gaman KCC and Mohou CHB. Studies of some therapeutic plants used in the treatment of high blood pressure and diabetes: two emerging diseases in Ivory Coast. Science & Nature, 2008; 5(1): 39-48.

41. Affy ME, Kouakou KR, Douhoure GT, Kouakou K.- Acute and subacute toxicity of the aqueous extract of *Amaranthus viridis* (Amaranthaceae) leaves in rats. J. Phyto. 2018; 7(4): 366-372.

Annexe 1 : Liste des trois plantes antimicrobiennes recensées à Dabou: caractères botanique et ethnobotanique

Appendix 1

N°	Species	Vernacular names (Adioukrou)	Family	Morphological type	biological Type	Phytogéographic Type	Part used	Mode of préparation	Indication Thérapeutique	Route of d'administration	recipe	Class
1	*Macaranga hurifolia* Beille	Librébr-sou	Euphorbiaceae	Tree	Microphanérophyte	GC	Leaf, Bark, stem	Ma, ex	To, T, œd, AGI (AR, AD,Ade)	Vo	PS	Dicotylédone
2	*Mallotus oppositifolius* (Geisel.) Müll. Arg.	Tchahan-egbe		Shrub		GC-SZ	Leaf, stem	Ex, dé	Pl, Brul, D (Ade)	Vc	MS	
3	*Mareya micrantha* (Benth.) Müll. Arg.	Hôre		Tree		GC	Leaf	Pé, dé	G, R, To	Vc, Vo	BS	

GC : Guinéo-Congolais ; GC-SZ : Guinéo conglais - Soudano-Zambézienne

PS : Multispecific ; MS : Monospecific ; BS : Bispecific

AGI=gastrointestinal disorder; D= diarrhea; G= scabies; To= cough; T=tuberculosis; Rhu= cold; Oed = edema; Pl=wound; Brul = burn

Ma: maceration; dé: decoction; Ex: Expression; Pé: Kneading; Vo: oral route; Vc: cutaneous route;