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Monograph: Acute Toxicity, Phytochemical Analysis and Ethnomedicinal Study of Aqueous (Leaf and Stem Back) Extracts of three Ethnomedicinal plants (*Macaranga hurifolia*, *Mareya micrantha*, and *Mallotus oppositifolius)* from Ivorian Pharmacopoeia on Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Macaranga hurifolia (ZG02), *Mallotus oppositifolius* (ZG04), and *Mareya micrantha* (ZG08) are ethnomedicinal plants belong to Euphorbiaceae family Which are frequently used in treating various diseases in 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 guidelinesThe rats were divided into fifteen groups of three rats each, 4 control groups,4ZG02 groups, 4 ZG04 groups, and 3 ZG08 groups).

Results of phytochemical analysis revealed that ZG02 contains polyphenols, flavonoids, alkaloids, gallotannins, and saponins. ZG04 contains polyphenols, flavonoids, catechin tannins, saponins, as well as sterols and terpenoids.While ZG08 contains 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 toxicity of ZG02 and ZG04, did not produce any mortality in the rats for dosages of up to 2000 mg/kg. Thus, the LD50 (lethal dose for 50% of the population) of ZG02 and ZG04 is greater than 2000 mg/kg of body weight. However, the treatment of ZG08 at 300 mg/kg of body weight, caused a modification in the behavior of the test animals, while the treatment at 2000 mg/kg of body weight resulted in the death of all 3 tested ras. The LD50 of ZG08 is equal to 500 mg/kg of body weight.

In conclusion, the presence of rich active phytochemical, justifying the 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.

1. INTRODUCTION

Knowledge of traditional medicine in herbal medicine has been passed down through generations and it plays 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, which enable them to treate various ailments successfully [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. Surprisingly, biological and chemical studies of 58% of these species are poorly understood [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 included analysis of the acute toxicity of the most commonly used plants in treatement of infections. It also provided crucial information that can enlighten traditional medical practices and potentially guide modern therapeutic approaches.

Using a multidisciplinary strategy that integrates ethnobotanical surveys, phytochemical analyses,

and acute toxicity evaluation, the aim is to add value to current traditional practices.

2. MATERIALS 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.

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 micrantha and 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 and dissolved in one liter of distilled water. The resulting homogenates were first squeezed through a clean white cloth, then successively filtered using hydrophilic cotton and Whatman filter paper no.3. Following filtration, the obtained filtrates underwent evaporation drying in a venticell-type oven set at 50°C. The resultant powders formed the complete aqueous extracts, identified and labeled as ZG02 (*Macaranga hurifolia*), ZG04 (*Mallotus oppositifolius*), and ZG08 (*Mareya micrantha*). They were then stored in sterile glass jars and kept refrigerated at 4°C until use.

2.3.2 Animal material

The animal study utilized 45 virgin nulliparous female rats (*Rattus norvegicus* strain Wistar), aged between 45 and 50 days and weighing 115 to 130 grams. The selection process followed the criteria outlined in paragraphs 11 and 12 of the OECD Guideline 423 (Organisation for Economic Co-operation and Development) [14]. These rats were housed in a controlled environment at a constant temperature of 24±2°C, under a 12-hour natural light and 12-hour dark cycle. The humidity levels were maintained between 50% and 55%, and the rats were provided with ad libitum access to both water and food (pellets

containing 15% protein and 4% fat) supplied by Faci-Abidjan. All protocols adhered to the guidelines stated in paragraph 13 of the OECD recommendations [14].

2.3.3 Acute toxicity method

Acute toxicity testing was performed in accordance with OECD Guideline 423 [14]. As outlined in paragraph 9, the extracts underwent sequential testing, involving three rats per batch at each stage for every plant extract.

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). After overnight fasting, 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

After receiving the extract, the animals underwent a subsequent period of fasting lasting 3 to 4 hours. Continuous monitoring of each animal occurred at least once within the initial 30 minutes, followed by regular observation throughout the initial 24-hour period. Particularly close attention was paid during the first 4 hours and daily for the subsequent 14 days post-extract administration. Comprehensive checks were conducted on all animals at least twice daily to detect any potential signs of pathology or alterations in behavior.

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:

If no more than one death occurred in the previous test, the same dose (300 mg/kg body weight) of the extract would be given to three additional animals as a repeat of the prior test. Alternatively, if there were zero or only one death in the previous tests conducted at a dose of 300 mg/kg body weight, the next step would involve administering an immediately higher dose (2000 mg/kg body weight) to three additional animals, constituting a test and repeat of the earlier test at the lower dosage. This latter test at 2000 mg/kg body weight would be repeated if no more than one death was recorded in a batch.

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 alkaloids were identified using two distinct reagents: Burchard's method employing the iodine-iodide reagent and Dragendorff's method utilizing potassium iodo-bismuthate reagent. For each, 6 mL of the solution underwent evaporation to dryness. Upon reconstitution with 6 mL of 60° alcohol, the addition of 2 drops of Dragendorff's reagent resulted in either a precipitate formation or an orange coloration. Subsequently, introducing 2 drops of Burchard's reagent to the alcoholic solution caused a reddish-brown precipitate, confirming a positive reaction. [15,16,17].

The reaction with ferric chloride ($FeCl_3$) was used to characterise polyphenols. A drop of 2% alcoholic ferric chloride solution was also added to two (2) mL of each extract (aqueous and 70% ethanol). The appearance of a more or less dark blue-black or green coloration was the sign of the presence of polyphenols [18].

Flavonoids were determined by the cyanidin reaction. Two (2) mL of each extract was evaporated and the residue was taken up in 5 mL of 2-fold diluted hydrochloric alcohol. The addition of 2-3 magnesium chips spurred a release of heat and a pinkish-orange or purplish coloration. The addition of 3 drops of isoamyl

alcohol intensified this coloration, which confirmed the presence of flavonoids. [19].

Saponins were identified based on their physical property: 10 mL of each aqueous extract was transferred into a test tube. After shaking the tube for 15 seconds, it was left undisturbed for 15 minutes. The observation of a persistent foam height exceeding 1 cm indicated the presence of saponins. [20].

Catechic tanins were tested using Stiasny's reagent. Five (5) mL of each extract was evaporated to dryness. After adding 15 mL of Stiasny's reagent to the residue, the mixture was kept in a water bath at 80°C for 30 minutes. The observation of a coarse flake precipitate characterised the catechic tannins. For the gallic tannins, the previous solution was filtered. The filtrate was collected and saturated with sodium acetate. The addition of 3 drops of FeCl3 caused the appearance of an intense blue-black coloration, which is a sign of the presence of gallic tannins [21,22].

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 sought by the Liebermann reaction. Five (5) mL of each of the two extracts (aqueous and 70% ethanolic) were evaporated on a sand bath. The residue was dissolved in 1 mL of acetic anhydride while hot and 0.5 mL of concentrated sulphuric acid was added to the triturate. The appearance of a purple or violet ring at interphase, turning blue and then green, indicated a positive reaction [16,17].

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 (Fig. 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].

Fig. 1. Leafy branches with inflorescences *Mallotus oppositifolius* (Geisel) Müll.Arg (Euphorbiaceae) [27]

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].

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 (Fig. 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.

Fig. 2. Leaves and leafy branch with inflorescences of *M. micrantha* (Benth.) Müll. Arg. (Euphorbiaceae). [27], (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 (Fig. 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.

The results of the phytochemical studies are recorded in Table 1. The phytochemical study of the aqueous extract of *Macaranga hurifolia* (zg02) has shown that, the phytochemical composition of the plant are polyphenols, flavonoids, alkaloids, gallotannins, and saponins. These findings corroborate Sylla *et al*. conclusions [37]**.** Similarly, the aqueous extract of *Mallotus oppositifolius* (zg04) revealed the presence of polyphenols, flavonoids, catechin tannins, saponins, sterols and terpenoids. It is also align with the discoveries of Pissang et al. [38]. The analysis of the aqueous extract from mareya micrantha (zg08) revealed the existence of polyphenols, flavonoids, gallotannins, catechin tannins, saponins, and sterols and terpenoids. Notably, alkaloids were not detected in zg08, which aligns with the prior discoveries made by Ladoh-Yemeda et al. regarding this particular 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.

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

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

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.

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 (TableS 2 and 3). However, treatment of ZG08 at 300 mg/kg body weight induced drowsiness, reduced mobility, and respiratory rate (Table 2). The dosage 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 (TableS 2 and 3).

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 dosage of 300 mg/kg BW, signs like drowsiness, reduced mobility, and decreased respiratory rate were observed. At the dosage 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 rats. These results suggested that the active compounds present in ZG08 can cause severe dysfunctions beyond the 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).

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*

4. CONCLUSION

The phytochemical study of the aqueous extracts of *Macaranga hurifolia, Mareya micrantha*, and *Mallotus oppositifolius* highlighted that, there are abundant active compounds, thus justifying their traditional use. The results of the acute toxicity study revealed that, *M. hurifolia* and *M. oppositifolius* have 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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standards written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX 1

N°	Species	Vernacular names (Adioukrou)	Family	Morphological type	Biological Type	Phytogéographic Type	Part used	Mode of préparation	Indication Thérapeu tique	Route of d'adminis tration	Recipe	Class
1	*Macaranga hurifolia* Beille	Librébr-sou	Euphor biaceae	Tree	Micropha nérophyte	GC	Leaf, Bark, stem	Ma, ex	To, T, œd, AGI (AR, AD,Ade)	Vo	PS	Dico tylédone
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;

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