

## *In vitro* cytotoxicity studies of sixteen plants used for pregnant women's health conditions in Menoua Division-West Cameroon

Didiane Mefokou Yemele<sup>1</sup>, Bruno Phelix Telefo<sup>1\*</sup>, Stephanie Chekem Goka<sup>1</sup>, Benoit Telesphore Nguenefack<sup>2</sup>, Pierre Simeon Chegaing Fodouop<sup>1,3</sup>, Richard Simo Tagne<sup>1,3</sup>, Landry Lienou Lienou<sup>1</sup>, Filomain Nguemo<sup>4</sup>, Paul Fewou Moundipa<sup>5</sup>

\*Corresponding author:

Didiane Mefokou Yemele

<sup>1</sup>University of Dschang, Faculty of Science, Department of Biochemistry, P.O.BOX 67, Dschang, Cameroon

<sup>2</sup>University of Dschang, Faculty of Science, Department of animal Biology, P.O.BOX 67, Dschang, Cameroon

<sup>3</sup>University of Ngaoundere, Department of Biomedical Sciences, P.O. Box 454, Ngaoundere, Cameroon

<sup>4</sup>University of Köln, Institute of Neurophysiology Robert-Koch Str. 39 D-50931 Köln, Germany

<sup>5</sup>University of Yaounde I, Faculty of Science, Department of Biochemistry, P.O.BOX 812, Yaounde, Cameroon

### Abstract

In Cameroon, many plants are used in traditional medicine for the treatment of pregnancy and childbirth complaints. However, toxicological potential of most of these plants have not been investigated. In order to evaluate the degree of safety of their users, *in vitro* cytotoxic potentials of sixteen of these medicinal plants were subjected to the assay using the brine shrimp lethality assay. From this study, the aqueous extract of plant *Rauvolfia vomitoria* bark was found to be cytotoxic and that of *Ageratum conyzoides* stem and leaves slightly cytotoxic, with LC<sub>50</sub> values of 17.62 and 99.17 µg/ml, respectively. The least toxic plant extracts were *Aloe buttneri*, *Commelina benghalensis*, *Ipomoea tenuirostris* and *Nelsonia canescens*, (LC<sub>50</sub> value > 10<sup>5</sup> µg/ml). Overall fourteen extracts were found to be non-toxic. Most herbal remedies were non cytotoxic but it would be necessary to complete these cyto-toxicological information by mutagenicity, teratogenicity tests as well as *in vivo* toxicological tests on animals.

**Keywords:** Cytotoxicity; Medicinal plants; Cameroon; Pregnancy; Maternal-infant health

### Introduction

Relegated for a long time to a marginal place in the health planning of developing countries, traditional medicine has undergone a major revival in the last twenty years. The importance of traditional medicine as a source of primary health care was first officially recognized by the World Health Organization (WHO) in the Primary Health Care Declaration of Alma Ata (1978) and has been globally addressed since 1976 by the Traditional Medicine Program of the WHO [1] Today about 80 % of the world population use herbal medicines for primary health care [2] because of their better acceptability and lesser side effects. In Africa, remedies made from plants play an important role in the health of millions of people. In Cameroon, medicinal plants are used for many sanitary problems and especially for pregnancy or childbirth complaints [3,4] which are every year responsible for the death of more than five hundred thousand women and four million new-born babies aged less than one month throughout the world [5,6].

In fact, for all aspects of woman reproductive health issues (menstruation, conception, pregnancy, lactation and menopause), a great number of plants species were used and continue to be

used, by women and tradipractitioners all over the world [7]. However, majority of these plants have not been investigated in spite of the fact that certain medicinal plants have been proved to have harmful side effects on the human being [8].

An ethnobotanical survey conducted in Menoua division (West-Cameroon) showed that many plants are largely used during pregnancy and/or childbirth in unlimited quantities and for a long period of treatment [4]. Given the fact that few and even no toxicological information are available for many of the recorded medicinal plants, it will be necessary to determine the degree of safety of their users. In order to minimize the poisonous effects and ensure safe utilization of natural products, the demand for research on their cytotoxic studies has been increased [9]

Brine shrimp lethality is a rapid general bioassay for identifying toxic dose of a compound. For the assessment of cytotoxicity, brine shrimp lethality assay is given preference over whole animal bioassays and cell line assays [10] because it have the advantage to be rapid, cost effective, no need of special equipment and animal serum and without any objection from animal right advocates to the use of these invertebrates for the experiment [11]. Thus the present proposal, which aims to evaluate the cytotoxic potentials of sixteen most used medicinal plants for the treatment

of pregnancy and childbearing ailments in some localities of the Menoua division (Western Region of Cameroon) by brine shrimp lethality assay for preliminary screening of their toxicity.

## Material and methods

### Collection of plant materials

Different fresh specimens of studied plants were collected from Dschang town or Bamendou village (Menoua Division, West-Cameroon), dried and stored in the laboratory of the Department of Biochemistry of the Faculty of Science of the University of Dschang. They were later identified at the Cameroon National Herbarium Yaounde where their full scientific names and voucher number were obtained.

### Extraction

Each dried sample was powdered and 100g macerated in 1l of distilled water at room temperature for 36hr. However, *Cymbopogon citratus* extract was prepared by decoction of 100g of dried powdered in 2l of distilled water for 30 min. Collected extracts were then filtered and concentrated at 45°C in hot air oven. The aqueous extracts obtained were kept in a refrigerator until used.

### Brine shrimp lethality assay

Brine shrimp (*Artemia salina* Leach) eggs were hatched in a beaker filled with sea water (32g of sea salt in 1l of distilled water)

under constant aeration. After 48 h eggs were hatched providing large number of larvae (nauplii). The nauplii were collected by pipette against a lighted background. Ten nauplii were transferred to each test tube containing the samples. Each sample was made by 5 ml of sea water containing the extract of plant or  $K_2Cr_2O_7$  (positive control) or Paracetamol (reference substance) at one of the eight following concentrations: 0; 1; 3; 10; 30; 100; 300; 1000 µg/ml. A live nauplii were counted in each tube after 24 h of incubation and the percentage of deaths at each dose was determined according to Meyer et al. in 1982 [12]. If control deaths occurred, the percent death values were corrected using Abbott's formula as expressed by Rasoanaivo and Ratsimamanga-Urverg in 1993 [13]. Test were carried out in quadruplicate and the experiments were repeated three times

### Data analysis

The probit of the mean results of brine shrimp mortality against the logarithms of concentrations were plotted using the Microsoft Excel program, which also gives the regression equations. The regression equations were used to calculate the 50% lethal concentration ( $LC_{50}$  values) and the efficacy index (EI) which is  $EI = \text{Concentration with maximal activity} / LC_{50}$ .

## Results and discussion

Table 1: List of the plants according to their medical use and Phytochemical compounds

Family of plant	Plant name	Medical use	Phytochemical compounds
Acanthaceae	<i>Eremomastax speciosa</i>	NR, CB, UI, SLA, FD, PAP, BP	Alkaloids, flavonoids, saponins and tannins [26]
	<i>Nelsonia canescens</i>	FD, CB, SLA, BS, ES	Alkaloids, tannins, flavonoids, and phenols [27]
Anthericaceae	<i>Aloe buttneri</i>	CB, SLA, AP, UI, PAP, CI, MP, FD	Glycosides, quinines, anthraquinonic derivatives and coumarins [28]
Apocynaceae	<i>Rauvolfia vomitoria</i>	NV, AP	Tannins, alkaloids, steroids, flavonoids, phenolic compounds and saponins [29]
Asteraceae	<i>Ageratum conyzoides</i>	SLA, F, Fo, NV, FD, BPB, PAP, RP, ES	Alkaloids, flavonoids, phenols, coumarins, sterols and terpenoids [30]
	<i>Crassocephalum bauchleuse</i>	CB, SLA, BPB	Alkaloids, phenols, tannins and sterols [31]
Commelinaceae	<i>Commelina benghalensis</i>	FD, CB, SLA, BP	Alkaloids, tannins, steroids, saponins and flavonoids [32]
Convolvulaceae	<i>Ipomoea tenuirostris</i>	FD, SLA, CB	
Cucurbitaceae	<i>Zheneria scabra</i>	CI, SLA	
Malvaceae	<i>Hibiscus noldeae</i>	UI, FD, PAP, SLA, CB, BP	
	<i>Hibiscus rosa sinensis</i>	FD	Alkaloids, phenols, steroids, flavonoids, triterpenoids, tannins, [3]
	<i>Sida veronicifolia</i>	SLA, FD, BS, PAP, BP, AP, CB	
Oxalidaceae	<i>Oxalis corniculata</i>	AP, SLA, CB, EF	Flavonoids, tannins, fatty acids, alkaloids, coumarins, phytosterols, glycosides, phenol, volatile oil and galactoglycerolipid [33]
Piperaceae	<i>Piper umbellatum</i>	FD, SLA, CB	Alkaloid, saponin, tannin and inulin [34]
Poaceae	<i>Cymbopogon citratus</i>	SLA, F, CB	Terpenes, phenols, saponins, anthraquinones, coumarins, tannins and flavonoids, [30]
Zingiberaceae	<i>Aframomum letestuanum</i>	PH, BP, ES, RP, FD, PAP, BPB, MP, SLA, CB, NV, Fo	

AP, abdominal pains; BP, bleeding during pregnancy; BPB, bad positioning of the baby; BS, body sweat; CB, cleaning of the baby; CI, cutaneous itching; ES, evil spirit; F, Fiber; FD, facilitation of delivery; Fo, Fortification; MP, muscular pains; NR nappy rash; NV, nausea and vomiting; PAP, postpartum abdominal pain; PH, postpartum hemorrhaging; RP, retained placenta; SLA, swelling of legs and ankles; UI, urogenital infections Source: [4]



Table 2: The brine shrimp lethality results represented as LC<sub>50</sub> in µg/ml and EI

Family of plant	Plant name	Part of plant used	LC <sub>50</sub> value (µg/ml)	Efficacy index
Acanthaceae	<i>Eremomastax speciosa</i> .	leaves	4 018.92	0.25
	<i>Nelsonia canescens</i>	whole plant	>10 <sup>5</sup>	can not be determined
Anthericaceae	<i>Aloe buttneri</i>	leaves	>10 <sup>5</sup>	can not be determined
Apocynaceae	<i>Rauvolfia vomitoria</i>	stem bark	17.62	17.03
Asteraceae	<i>Ageratum conyzoides</i>	stem and leaves	99.17	10.08
	<i>Crassocephalum bauchiense</i>	stem and leaves	20 971.12	0.05
Commelinaceae	<i>Commelina benghalensis</i>	whole plant	>10 <sup>5</sup>	can not be determined
Convolvulaceae	<i>Ipomoea tenuirostris</i>	whole plant	>10 <sup>5</sup>	can not be determined
Cucurbitaceae	<i>Zheneria scabra</i>	whole plant	955.26	0.31
Malvaceae	<i>Hibiscus noldea</i>	whole plant	20 535.25	0.05
	<i>Hibiscus rosa sinensis</i>	stem and leaves	102.66	0.97
	<i>Sida veronicifolia</i>	whole plant	385.84	2.59
Oxalidaceae	<i>Oxalis corniculata</i>	whole plant	165.90	6.02
Piperaceae	<i>Piper umbellatum</i>	leaves	20 154.98	0.05
Poaceae	<i>Cymbopogon citratus</i>	whole plant	128.37	2.34
Zingiberaceae	<i>Aframomum letestuanum</i>	fruit	359.25	2.78

LC<sub>50</sub>s were estimated using probit transformation. Therefore confidence intervals are not provided.

Plants are used in Africa for the treatment of many pregnancy and childbirth complaints. As shown in table 1, our 16 studied plants are used to cure 18 complaints and each plant except *Hibiscus rosa sinensis* is used in many treatment. These observations attest the wealth of African flora and could be attributed to the fact that, a single plant can contain many chemical compounds which perform different functions in the body [3].

In order to verify whether the medicinal plants used to cure pregnancy and childbearing complaints may have toxic effects on mothers or child, sixteen aqueous extract of plants were tested for brine shrimp lethality. Among the sixteen extracts tested, two were cytotoxic. These include *Rauvolfia vomitoria* extract which showed high toxicity to the shrimps with LC<sub>50</sub> values of 17.62 µg/ml and the extract of *Ageratum conyzoides* which was slightly cytotoxic with LC<sub>50</sub> values of 99,17 µg/ml. these two plants also showed the highest efficacy index of 17.03 And 10.08 respectively. The fourteen others qualified as non cytotoxic gave LC<sub>50</sub> values greater than 100 µg/ml (Table 1). It is noted that some of them have Similar results were obtained on *Rauvolfia vomitoria* by Zirihi *et al.* in 2005 [14] which prove that ethanolic extract of the plant obtained by decoction were cytotoxic. It has also been shown that *Rauvolfia vomitoria* has teratogenic potential on fetal heart [15]. Given the fact that teratogens are agent that, on embryonic exposure, induces or increases incidence of abnormal prenatal development which can induced the death or the malformation of the embryo [16]; the teratogenic potential of *Rauvolfia vomitoria* may be the cause of his cytotoxicity. Consequently the plant extract may be dangerous for the fetus if it is consumed by pregnant women.

The study of chronic toxicity made on ethanolic extract of *Ageratum conyzoides* by Sumalatha in 2012 [17] proved that it is shightly toxic when administrated for long time. Other study made by Diallo *et al.*, 2014 [18] revealed that the hydroalcoholic leaf extract of *Ageratum conyzoides* is cytotoxic and can induce liver, kidney and haematological disorders when it is administered in higher doses

for 90 days to the rats. These results are in conformity with ours finding. Then *Ageratum* may be effectively lightly cytotoxic.

For others studied plants which were found to be non cytotoxic, literature on some of them confirmed our results while no report was obtained on other. For the former it was proved that: the methanolic extract of *Zheneria scabra* is non toxic [19]; the aqueous extract, alkaloid fraction, ethyl acetate extract and fractions of *Crassocephalum bauchiense* did not exhibit any acute toxicity [20,21]; the hydroethanolic extract of *Piper umbellatum* is non cytotoxic and without any acute toxicity [22]; the methanolic flower extract of *Hibiscus rosa sinensis* has no genotoxic activity [23]; the ethanolic extract of *Oxalis corniculata* may safely be used at lower doses for therapeutic purposes [24]; the leaf methanolic extract of *Aloe buettneri* is without any acute toxicity and has toxic effects only in the case of prolonged intake of high doses of the extract [25]. These informations proved the reliability of our results.

## Conclusion

This study showed that aqueous extracts of *Rauvolfia vomitoria* bark and *Ageratum conyzoides* stem and leaves were cytotoxic while the rest of studied plants were relatively non toxics. However more works are needed in order to determine their usefulness and their toxicity on animals which will be crucial as a way to definitively judge the safety of these plants.

## Authors' Contributions

DMY: Prepared the extract, carried out the assays and drafted the manuscript. BPT: Supplied certians materials, coordinated the study and refined the manuscript. SCG: Helped in the experimental work. BTN: Supplied certians materials, coordinated the study. PSCF: Supplied the materials, and refined the manuscript. RST:



Helped in the experimental work. LLL: Helped in the experimental work. FN: Supplied the materials, PFM : Supervised the work

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