In vivo Antidiabetic Activities of Aqueous Extract of Anchomanes difformis (Blume) Eng, Rhizophora racemosa G. Mey and Ravenala madagascariensis Sonn

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Authors’ contributions

This work was carried out in collaboration among all authors. Author AKC designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author ESB wrote the protocol, performed the statistical analysis and review the first draft of the manuscript. Author MH managed the analyses of the study. Author AC managed the literature searches. Author KG collected and identified the plants samples. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Anchomanes difformis (Blume) Eng (Araceae), Rhizophora racemosa G. Mey (Rhizophoraceae) and Ravenala madagascariensis Sonn (Strelitziaceae) are used in traditional medicine in Guinea for diabetes management. The aim of this work was to test the antidiabetic activity of these plants and to determine their toxicity.
Material and Methods: Extemporaneous extracts based on these plants were tested for their acute toxicity, their effects in normoglycemic rats and rendered hyper glycemic by the oral route in comparison with the glibenclamide an antidiabetic agent. Swiss albino mice and Male Wistar rats were used respectively for acute oral toxicity and antidiabetic activity.

Results: This study showed that the administration of the 2000 mg / kg dose of dry extracts in mice showed no acute toxicity and adverse effect. At a dose of 400 mg/kg these three plants showed significant hypoglycaemia effect. The average blood glucose levels ranged from 111.2 ± 6.2 to 93.2 mg/dL (p<0.001) for A. difformis, 110.6 ± 6 to 93.2 mg/dL (p<0.05) for R. racemosa and 99.6 ± 14.4 to 82.3 mg/dL (p<0.05) for R. madagascariensis.

Conclusion: The results obtained on the antidiabetic properties of these three plants corroborates their traditional uses in the management of type 2 diabetes in the Republic of Guinea.

1. INTRODUCTION

Human exploitation of plant diversity in the provision of medicine follows two main strategies: random screening guided by taxonomy where chemotaxonomy draws on the phylogenetic relatedness in the search for identical or similar bioactive compounds, or ethnopharmacological approaches from therapeutic indications of indigenous drugs [1]. Medicinal use of herbal medicines in the treatment and prevention of non-communicable diseases, including diabetes and hypertension, has a long history compared to that of conventional medicine [2]. Due to the side effects of conventional drugs, attention is now focusing on the development of nano-sized herbal drugs and their precise and targeted delivery, which may help to overcome the limitations and complications of conventional drugs used to control diabetes mellitus [3].

Ethnopharmacological surveys have indicated that more than 1,200 plants are used worldwide in traditional medicine for their alleged hypoglycaemic activity [4-12], like Anchomanes difformis ENGL, Anthocleista vogelii Planch, Garcinia lancifolia Roxb, Ravenala madagascariensis Sonn, Zanthoxylum armatum DC. Management of diabetes using traditional remedies is widespread in Africa in rural as well as urban communities. An increasing number of patients opt for traditional remedies driven by a combination of factors, including financial constraints and/or geographical accessibility of the population to orthodox antidiabetics, the inadequacy of the health care system, ease of accessibility of traditional medicine, indigenous knowledge by community members and the role of traditional healers [13-14].

In the Republic of Guinea, previous ethnobotanical investigations indicated a wide used of plant species in the management of diabetes, particularly type 2 diabetes. And, the relatively low cost of treatment and easier access to medicinal plants could explain the popularity of herbal medicines [15-17].

Among the listed plants Anchomanes difformis (Blume) Engl (Araceae), Rhizophora racemosa G. Mey (Rhizophoraceae) and Ravenala madagascariensis Sonn (Strelitziaceae) feature prominently. A. difformis, R. racemosa and R. madagascariensis are plants with several reported therapeutic effects, and they are used in many parts of Africa for the traditional management of malaria, asthma, diabetes, antimicrobials [18-23].

This study aimed to evaluate the antidiabetic activity of these three plants, on Male Han Wistar rats and their toxic effects on swiss albino mice.

2. MATERIALS AND METHODS

2.1 Herbal Materials

Voucher specimens were collected, and botanical identification was conducted by Karman Guilavogui (a botanist at the National Herbarium of Guinea). The voucher specimens of Anchomanes difformis (Blume) Engl. (HNG002577), Rhizophora racemosa G. Mey (HNG002578) and Ravenala madagascariensis Sonn (HNG002579) were deposited at the National Herbarium of Guinea.

The petioles of R. madagascariensis were harvested in the Camayenne area (Conakry); the root bark of R. racemosa was harvested in the
Yimbaya area (Conakry), and the tuber of *A. difformis* was harvested in Coyah (50 km from Conakry). The samples were dried in the drying room of the Pharmacognosy Laboratory of the Pharmaceutical and Biological Sciences Training Unit of the University of Conakry after a period of 45 days and were then sprayed.

### 2.2 Animal Material

Male Wistar rats weighing between 150-215 g were used for the antidiabetic activity evaluation and female Swiss albino mice weighing between 22-28 g were used for the acute toxicity evaluation.

Animals were maintained in standard and constant laboratory conditions (23 – 25 °C and light/dark cycles, i.e., 12/12 h). The animals were fasted for 16 h, weighed and marked, and had free access to water before the start of the tests.

### 2.3 Methodology

#### 2.3.1 Preparation of plant extracts

Five grams of each powdered sample was infused into 200 mL of boiling distilled water for 15 min. Infusions were filtered and cooled in the refrigerator.

#### 2.3.2 Acute oral toxicity study

The acute oral toxicity study was conducted using test guidelines on acute oral toxicity (test 423) according to the Organization for Economic Cooperation and Development and as described by Alanin [20,22,23,19,18].

Mice were randomly divided into four groups of three female mice and treated by the oral route with a single dose of *A. difformis*, *R. racemosa*, *R. madagascariensis* or water. For *A. difformis*, *R. racemosa*, and *R. madagascariensis* a dose of 2,000 mg/kg (corresponding to 50 mL/kg) was administered, while 20 mL/kg was used for water treatment. Animals were observed for the first 4 h after treatment to record the immediate death and once daily for 14 days to record any manifestation of toxicity.

#### 2.3.3 Evaluation of the antidiabetic activity

##### 2.3.3.1 Assay in normoglycemic rats

In control animals, the average blood glucose level was 98.4±7.4 mg/dL and 85.8±6.7 mg/dL.
after the oral administration of 10 mL distilled water, with 0 h values considered the control.

Oral administration of the aqueous extract *A. difformis, R. racemosa* and *R. madagascariensis* at the dose of 10 ml/Kg (400 mg/kg) body weight produced significant hypoglycaemia effect (Fig. 1). The average blood glucose levels ranged from 111.2 ± 6.2 to 84.6 ± 6.7 mg/dL (T0 – T3; p<0.001) for *A. difformis*, 110.6 ± 6 to 93.2 mg/dL (T0 – T3; p<0.05) for *R. racemosa* and 99.6 ± 14.4 to 82.3 mg/dL (T0 – T3; p<0.05) for *R. madagascariensis*.

### 3.3 Antihyperglycemic activities of aqueous extracts of *A. difformis, R. racemosa* and *R. madagascariensis*

At the dose of 400 mg per kg body weight (10 ml/Kg), the lowering of blood glucose levels was significant (p < 0.001) in experimental animals compared to blood glucose levels in the control group at the time intervals of 30, 60, 120 and 180 min (Fig. 2).

In all groups, the glucose peak was observed 30 min after hyperglycaemia induction. The mean blood glucose levels obtained were 158.6 ± 18.7 mg/dL 30 min after overload and 81.2 ± 16.8 mg/dL 3 h after overload for the *A. difformis* extract, 138 ± 4.4 mg/dL 30 min after glucose challenge and 105.6 ± 12.3 mg/dL 3 h after administration of the *R. racemosa* extract, and 86.2 ± 8 mg/dL at time zero and 74.2 mg/dL 3 h after glucose overload of the *R. madagascariensis* extract. Excepted for the group 1 (distilled water), the glycaemia value regression started on time T30 and no significant changes of blood glucose levels were observed in normal-glycemic rats treated with the three plant extracts or glibenclamide at a dose of 5 mg/kg body weight during the 3 h of the experimental period.

![Fig. 1. Hypoglycaemia activity evaluation of aqueous extract *A. difformis, R madagascariensis, R racemosa*](image_url)
4. DISCUSSION

The observed data suggested that the oral LD50 values of *A. difformis*, *R. racemosa* and *R. madagascariensis* are greater than 2000 mg/kg in Swiss albino mice. Therefore, according to the OECD Globally Harmonized Classification System [24] extracts from *A. difformis*, *R. racemosa* and *R. madagascariensis* are categorized as category 5 (2000 mg/kg < LD50 < 5000 mg/kg) and considered non-toxic orally. The low toxicity of these plants has been confirmed by other authors in various studies [25,26]. We found that the innocuity of *A. difformis*, *R. racemosa* and *R. madagascariensis* is in concordance with the wide use of these plants in traditional medicine [19,21,22,27].

The hypoglycaemic activity of the extemporaneous extract of *A. difformis* revealed an effect on basal glucose at the third hour after administration. We found that glycaemia should be monitored when the plant is used. The aqueous extract of *A. difformis* effectively decreased blood glucose after hyperglycaemia induction in rats. However, in the control group, hyperglycaemia persisted at the end of the experiment. Our data suggest that *A. difformis* possesses potential antidiabetic activity. In a previous study, ethanolic extracts of the leaves and roots of *A. difformis* at doses of 250 mg/kg and 500 mg/kg demonstrated significant decreases in hyperglycaemia in albino rats [10,11]. Phytochemical screening of *A. difformis* revealed the presence of flavonoids, saponins, and tannins and suggest that the plant is very rich in antioxidant compounds [25]. Antioxidant compounds are responsible of protective roles of vegetables and plant foods against diabetes and other neurodegenerative diseases.

The hypoglycaemia activity of the extemporaneous extract of *R. racemosa* revealed an effect on the basal glucose at the third hour after administration. This shows that glycaemia should be monitored when the plant is used. The data revealed that the *R. racemosa* aqueous extract induced a significant decrease
in hyperglycaemia after induction, thus corroborating the potential antidiabetic activity of the plant. The antidiabetic activity of *R. racemosa* reported in Douala was 250 mL of decoction 3 times daily: 2 g of stem bark per kg of body weight should be boiled in 2 L of water for 15 min to drink [28]. Revathi et al. reported that the flowers and leaves of *R. racemosa* contain compounds with antidiabetic activity [29]. Cinchonain Ia, Cinchonain Ib, Catechin-3-O-rhamnopyranoside, Epicatechin, Lyoniside and nudiposide were found to be in *R. racemosa and could be associated to the hypoglycemic effect of *R. racemosa* [30].

The hypoglycaemia activity of *R. madagascariensis* evaluated at the dose of 10 mL/kg caused a non-significant effect on basal glucose at the third hour after administration. At this same dose, the antihyperglycaemia activity was also evaluated, and we found a significant decrease in blood glucose levels in the rats for the first hour, and the antihyperglycaemic effects persisted until the end of the experiment. Priyadarsini et al. reported that administration of both ethanolic and aqueous leaf extracts of *R. madagascariensis* produced a significant reduction in blood glucose levels in a dose-dependent manner. The ethanolic extract was found to be more effective than the aqueous extract, which may be due to the higher solvent extraction ability of ethanol compared with water [6]. Cycloartanol, a triterpene with antidiabetic activity was found to be in *R. madagascariensis* [31].

5. CONCLUSION

Achieving correct diabetes care without side effects is a challenge for the research community. This study confirmed the therapeutic efficacy of aqueous extracts of the tubercle of *A. difformis* (Blume) the root bark of *R. racemosa*, and the petioles of *R. madagascariensis* in hyperglycaemic rats. The results obtained on the antidiabetic properties of these three plants corroborates their traditional uses in the management of type 2 diabetes in the Republic of Guinea.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal experiments were conducted at the Department of Traditional Medicine of the National Institute of Public Health Research in Bamako, according to the local ethical committee of the institution.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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