The Journal of Phytopharmacology 2017; 6(5): 260-263
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2017; 6(5): 260-263
September- October
Received: 23-09-2017
Accepted: 24-10-2017

Morabandza Cyr Jonas
Biochemical and Pharmacological Laboratory, Health Sciences Faculty, Marien NGOUABI University, P.O.Box 69, Brazzaville-CONGO

Nkounkou Loupangou Celestine
a) Unity of Vegetable chemical and life, Sciences and Technical Faculty, Marien NGOUABI University, P.O.Box69, Brazzaville-CONGO
b) Departments of Exact Sciences, E.N. S, MarienNGOUABI University, P.O.Box 69, Brazzaville-CONGO

Etou Ossibi Arnaud Wilfrid
Biochemical and Pharmacological Laboratory, Health Sciences Faculty, Marien NGOUABI University, P.O.Box 69, Brazzaville-CONGO

Ongoka Pascal Robin
Departments of Exact Sciences, E.N. S, MarienNGOUABI University, P.O.Box 69, Brazzaville-CONGO

Ouamba Jean Maurille
Unity of Vegetable chemical and life, Sciences and Technical Faculty, Marien NGOUABI University, P.O.Box 69, Brazzaville-CONGO

Abena Ange Antoine
Biochemical and Pharmacological Laboratory, Health Sciences Faculty, Marien NGOUABI University, P.O.Box 69, Brazzaville-CONGO

Correspondence:
Morabandza Cyr Jonas
Biochemical and Pharmacological Laboratory, Health Sciences Faculty, Marien NGOUABI University, P.O.Box 69, Brazzaville-CONGO
Email: cyrmoras[ay]yahoo.fr

In vitro immunitary impact and antioxidant activity of aqueous extracts of *Maprounea africana* Müll (Euphorbiaceae) and *Mitragyna stipulosa* O.Kze (Rubiaceae)

Morabandza Cyr Jonas, Nkounkou Loupangou Celestine, Etou Ossibi Arnaud Wilfrid, Ongoka Pascal Robin, Ouamba Jean Maurille, Abena Ange Antoine

ABSTRACT

This study aims to investigate the *in vitro* immunitary impacts and antioxidant activity of aqueous extracts of *Maprounea africana* (Euphorbiaceae) leaves and *Mitragyna stipulosa* (Rubiaceae) stem barks. Impact on leukocyte cells (total lymphocyte, polynuclears, monocyte, NK, TCD8 and TCD4) was quantified by using flow cytometry and, antioxidant activity by quantification of hydrogen peroxide production after immuno-marking of specific monoclonal antibodies. The results showed a significant descrease of total lymphocyte, polynuclear, NK, TCD8 and, a non-significant descrease of TCD4 and monocyte induces by aqueous extract of *M. africana* leaves. Whereas aqueous extract of the stem bark of *M. stipulosa* induces a significant increase of total lymphocyte, TCD4, NK, TCD8 and, a significant descrease of polynuclear and monocyte. The two extracts significantly reduce ($p<0.001$) the production of hydrogen peroxid by polynuclear, lymphocytes and monocytes. These results suggest an immunomodulatory and immunostimulant effect of *M. africana* and *M. stipulosa* respectively and, antioxidant activity. The present study established pharmacological evidence to support traditional uses of these two species and may open up the possibility of finding the new compounds against immunological desseases.

Keywords: *Maprounea africana*, *Mitragyna stipulosa*, Immunitary impact, Antioxidant.

INTRODUCTION

Oxidative stress is currently accused in the release and the progression of several diseases. Indeed, the production of free radicals by certain organism's reactions join with external factors causes, their overproduction leading to irreversible cellular damage [1]. These free radicals lower the blood level of glutathion, principal antioxidant system of organism defense, follows then a weakening of immunity, installation of immunitary deficits and others pathologies [2]. However, in clinical practice, these diseases have few specific remedies. Facing the considerable increase of these pathologies, the treatment with anti-cancer and immunostimulants drugs become a real public health problem because not only of their insufficiency and their inaccessibility, but also their important undesirable secondary effect son organism [3]. That is, the reason why there is urgent necessity to research and to develop a new drug accessible to all. Recent studies revealed that *Echinacea purpurea* would stimulate the production of leukocyte and interferon gamma in blood [4, 5]. Other studies seem to indicate that, vegetable extracts would stimulate the production of monocytes and modulate immune system function [6, 7, 8]. These results, let think that immunitary reaction could be improved by using vegetable extracts. Thus, in agreement with this hypothesis, we were interested by *M. africana* Müll (Euphorbiaceae) and *M. stipulosa* (Rubiaceae), two species used in Congolese traditional medicine against severals pathologies with immunitary components [9]. A former study had already highlighted stimulative activity of *Mitragyna inermis* on immune system in rodent [10]. Another recent study revealed that *M. africana* would present anti-cancerous properties [11]. Thus, the present work aimed to evaluate immunitary impact and antioxidant activity of aqueous extracts of *M. africana* and *M. stipulosa*.

MATERIALS AND METHODS

Plants collection

The leaves of *M. africana* and the stem barks of *M. stipulosa* were collected in April 2005 in the southern of Brazzaville. They were dried at the temperature of laboratory and pounded into powder

using a wooden mortar.

Plants extraction

100 g of powder of leaves and barks, respectively of *M. africana* and *M. stipulosa* were boiled during 30 mn in 1000 ml of distilled water. The obitaine decocted were filtered with the filter paper Macherey-Nagel and, evaporated by rotavapor at 60 °C. The extracts are freeze-dried using lyophilisator ALPHA.1-2 LD Christ GMBH for 48 h at -65°C and 0.015 mbar of pressure. The obtained yield were 10.2 % for *M. stipulosa* and 23.3 % for *M. africana*. The powders were preserved from humidity tightly closed bottles.

Experimental animals

Albinos male mice weighing between 35 and 40 gfrom Ethnobotanical and Pharmacological laboratory of University of Metz (France) were used for this study.

Immunitary impact

In order to evaluate immunitary impact of the two extracts, 7 groups of 4 male mice each one were treated orally as follows: one group served as controland treated with distilled water at 0.25ml/100g, three groups treated with aqueous extract of *M. maprounea* and three groups with aqueous extract of *M. africana*, respectively at 50, 100 and 200 mg/kg. The mice were anaesthetized with halothane vapor in an enclosure seal 24 hours after treatment, then sacrificed by decapitation and, blood collected in heparinized tubes.100µl of heparinized blood was distributed in several Epenndorf tubes to which each one was added 5 µl each solution of specific monoclonal antibodies (Nk-fitc, Cd8-EP, CD4-PerCP) to each leucocytic population. The tubes were homogenized by vortexing and incubated for 15 min in darkness.2ml of diluted ($1/10^{th}$) lysis solution was added in each tube to lyse the red blood cells. After homogenisation by vortexing, the sample were placed in darkness for 10 minutes. All tubes were centrifuged at +4°C for 5 min at 2000 rpm and the supernatant was eliminated. Each base was washed in 2ml of PBS. After washing each base was included in 500µl of PBS. The contents were transvased directly in tubes with hemolyze for flow cytometry. The percentage of cells of each leucocytic population was determined and compared with that of the control.

Antioxidant activity

Antioxidant activity of *M. afriacana* and *M. stipulosa* extractswas evaluatedafter immuno marking by specific monoclonal antibodies. Hydrogen peroxide (H_2O_2) production were quantified by using 2',7'-diacetat dichlorofluorescin (DCFH-DA). This substance which crosses membrans, was hydrolized by cytosolic esterases in dichlorofluorescin (DCFH), non fuorescent compound. In the presence of H_2O_2, the DCFH was oxidized in 2',7'- dichlorofluorescin (DCF) strongly fluorescent substance. Plant extract which involved the reduction of fluorescence intensity would be considered as having antioxidant potentiality.

Animals treatment and preparation of collected blood

Mice were anaesthetized with halothane and sacrificed by decapitation using the guillotine to increase thepossible quantity of blood collected in eppendorf tubecontaining three heparin drops. 9groups of 5 tubes tubes each one; containing collected blood were used as follow: onegroup asnegative control, tubes used to evaluate basic intracellular hydrogen peroxide rate; onegroup as positive control, tubes to which the hydrogen peroxide was added; 3 groups of tubes treated with aqueous extract of *M. africana* and *M. stipulosa*; respectively at the doses of 50, 100 and 200 mg/kg; one group of tube treated with chlorogenic acid at the dose of 0.5 mg/kg.100 µl of heparinized blood were put in each tube to which 2 ml of lysis solution diluted at $1/10^{th}$ were added. The tubes weremixed by vortexing and incubated for 10 min in darkness. After centrifugation at 4°C during 5 min at 2000 rpm, the supernatant was eliminated and 2 ml of PBS solution added. The tubes were agitated thereafter by vortexing and centrifuged under the same conditions as previously. After elimination of the supernatant, 1ml of washing PBS solution was added again in each tube with 5µl of DCFH-DAsolution.5µl of extract of *M. africana* and *M. stipulosa* at studied doses is respectively added and 5 µl of chlorogenic acid solution at 0.5 mg/kg with the last group of tubes. The tubes were again mixed by vortexing then placed for15 min in a drying oven at 37°C. After addition of 5 µl of hydrogen peroxide solution at 89 Mm in all tubes, excepted negative control tubes; the tubes are mixed by vortexing again and placed for 15 min in drying oven at 37°C before successively transvasing the contents of each tube in tubes with hemolyse for flow cytometry.

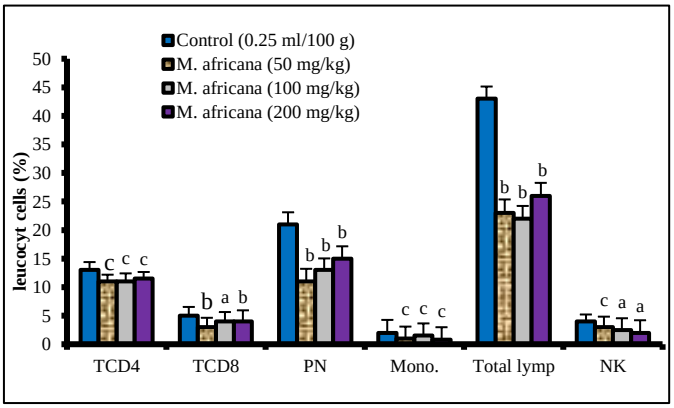
Statistical analysis

The results expressed affected on average of the standard error are submitted to an analysis of the variance to a factor followed of Student-Ficher test. All tests were considered statistically significant when $p<0.05$.

RESULTS

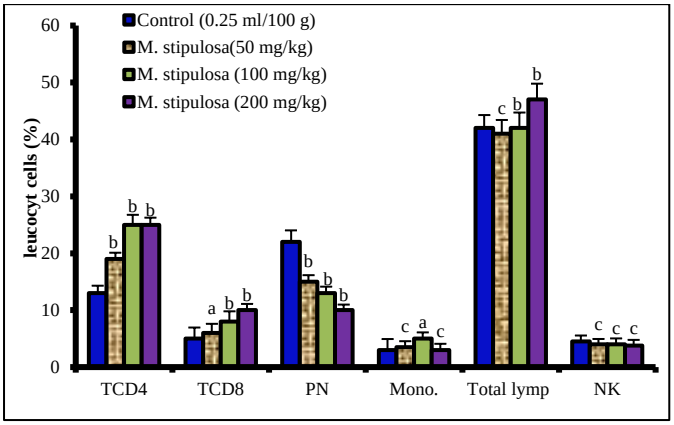
Immunitary impact

Figures 1 and 2 presents the results of immunitary impact of aqueous extracts of the leaves of *M. africana* and the stem barks of *M. stipulosa* (50, 100 and 200 mg/kg). At the studied doses, the extract of *M. africana* leaves induces a significant descrease of total lymphocyte, polynuclear, TCD8 and the one of NK at 100 and 200 mg/kg particularly. On the other hand one observes a nonsignificant descrease of TCD4 and monocytes rate compared to the control (figure 1). In figure 2, the extract of *M. Stipulosa* induced a significant increase of total lymphocyte, TCD4 and TCD8 without modifying significantly the rates of NK cells. One observance however a significant reduction of polynuclear and monocytes compared to the control.



(n=4) a= p<0.01, b=p<0.001 c= non significant

Figure 1: Effect of aqueous extract of *Maprounea africana* leaves on leucocyt cells rate



(n=4) a= p<0.01, b=p<0.001 c= non significant

Figure 2: Effect of aqueous extract of stem bark of *Mitragyna stipulosa* on leucocyt cells rate

Antioxidant activity

Table 1 present the results of antioxidant activity of aqueuos extracts of *M. africana* leaves and *M. stipulosa* stem barks. The two extracts reduce significantly (p<0.001) the production of hydrogen peroxide by polynuclear, lymphocte and the monocyte compared to the uses control (cholorogenic acid). This reduction is more noticed with the extract of *M. africana* at the dose of 200 mg/kg.

Table 1: Effect of aqueous extract of *M. africana* and *M. stipulosa* on Hydrogen peroxide rate(%) of lymphocyts, polynuclears and monocyts

Treatment	Doses (mg /kg)	Hydrogen peroxide rate (%)		
		Lymphocyts	Polynuclears	Monocyts
Control (-)	1 ml H_2O/100	1.70 ± 0.60	1.60 ± 0.20	01.40 ± 0.38
Control (+)	1 ml H_2O/100	95.20 ± 1.40	96.65 ± 1.10	87.32 ± 1.40
M. africana	50	62.46 ± 0.40**	69.44 ± 0.20*	54.02 ± 0.70**
	100	13.74 ± 0.30***	12.20 ± 0.10***	7.40 ± 0.60***
	200	1.89 ± 0.50***	3.19 ± 0.30***	3.28 ± 0.10***
	50	48.75 ± 0.10**	65.36 ± 0.30*	68.85 ± 0.08*
M. stipuosa	100	38.99 ± 0.06***	59.30 ± 0.20**	43.95 ± 0.50**
	200	53.85 ± 0.10**	63.10 ± 0.09**	61.39 ± 0.30*
Chloro Ac	0.5	7.61 ± 0.20***	5.74 ± 0.20***	3.50 ± 0.10***

Values are expressed as mean ± standard error of mean (SEM, n=4); *p<0.05; **p<0.01; ***p<0001.

DISCUSSION

The object of this study was to evaluate *in vitro* immunitary impact and antioxydant activities of aqueous extracts of *M. africana* leaves and *M. Stipulosa* stem barks in male mouse.

From figure 1, it come out that aqueous extract of *M. africana* leaves induced at the studied doses a significant descrease of some under leucocytic populations: lymphocytes total, polynuclear, NK and, of the TCD8.This would indicate a immunosuppressor effect. The extract does not induce however a significant descrease of TCD4 and monocyte. Indeed TCD4 and monocytes are immunitary cells playing a very important role in the immunecellular response and humorale mediation. In this experimentation, although nonsignificant, their descrease is unfavourable for immune defenses; the two combined effects rather suggest a probable immunomodulator effect of aqueous extract of *M. Africana* leaves. At the same doses and in the same conditions, the extract of *M. Stipulosa* stem barks induces a significant increase of total lymphocyte, TCD4 and TCD8 without modifying significantly the rates of NK cells with however a significant descrease of polynuclear and monocyte. The two latest cells are leukocytic populations which during the migration towards immune reaction center produce free radicals: dangerous compounds in organism [1]. Thus, the significant increase in some leukocityc populations and the significant descrease of polynuclear and monocyte would let think of a immunostimulant purpose. This fact would explain the abundant traditional use of this species. The extract of the stem barks of *M. stipulosa* could contribute to increase the capacity of defense against infectious diseases and, to reinforce the immune system [12, 13]. During physiological and immune reactions, the migration of lymphocyte, polynuclear and monocyte are always accompanied bythe production of free radicals [14]. This production causethe descrease of glutathion blood level, principal antioxydant system of organism defense. This low level would inhibit the proliferation, growth and the differentiation of immune cells. An analysis of the results of effects of the two extracts on immunitary impact suggest the presence in these extracts of some compounds endowed with protection capacity of immune cells by neutralization free radicals. That is why it is necessary to evaluate the rate of hydrogen peroxidproduction. The obtained results in table 1 revealed a significant descrease of hydrogen peroxide rate by the aqueous extracts of *M. africana* and *M. stipulosa* showing their antioxydant capacity. These results are in agreement with our earlier results showing impact of polynuclear and monocyts recognized asfree radicals (hydrogen peroxid) producing cells. The two extracts induced significant reductions of these cells (figures 1 and 2). This antioxidant effect would be probably explained by the presence in these extracts of compounds endowed antioxydant potentialities (polyphenols, vitamin E) able to neutralize free radicals, to slow down the destruction of defense cells and, to contribute thus to the prevention of certain pathologies as cancer [15].

CONCLUSION

The results of this study showed that the aqueous extract of the leaves of *M. africana* presents a immunomodulator effect whereas that of the stem barks of *M. stipulosa* would present a immunostimulant effect. The results of this study would probably explain abundant use of these two species in traditional medicine. However, a phytochemical and biological study with more elaborate models deserves to be carried out in order to identify the active ingredients implied in the effects observed.

REFERENCES

1. Durackova Z, Djrolo F, Houngbe H, Avode G, Attoulou V, Addra B, *et al*. Oxidants, antioxidans and Oxidative stress. *Mitochondrial medicine. Gvozdjakova A ed*, 2008; 19-40.
2. Urban BC, Roberts DJ. Malaria, monocytes, macrophages and myeloid dendritic cells: sticking of infected erythrocytes switches of host cells. *Current Opinion in immunology* 2002; 14:458-465.
3. Blétry O, Kahn JE, Somogyi A. Immunologie Réaction inflammatoire. *Masson 2ème édition*, 2006; 8:225.
4. Hudson JB. Applications of the phytomedecine Echinacea purpurea (Purple Coneflower) ininfectious diseases. Journal Biomed Biotechnol. 2012; 769-896.
5. Storage Ugal. Com/5351/ *Echinacea*. Pdf. *Echinacea purpurea*, *Moenc Echinacea angustifolia*, De Candolle *Echinacea pallida*, *Natull Astéracées* visited 21/10/2013, 13 hour 57 mn.
6. Sakagami H, Sakagami T, et Takeda M. Antiviral properties of polyphénols. *Polyphenols Actualities*,1995; 12:30-32.

7. Middleton EJ. Biological properties of plant flavonoids: an overview. Int. J. Pharmacol 1996; 34(5):344-348.
8. Bachelet B. Impact de la phytothérapie sur le système immunitaire. Thèse de doctorat, Facultéde médécine Creteil, France, 2013; 137p.
9. Adjanohoun EJ, Ahyi AMR, Aké Assi L, Baniakina J, Chibon P, Cusset G, *et al*. Contribution aux études ethnobotanique et floristiques en République du Congo *ACCT*, Paris, 1988; 161-165.
10. Ouedraogo Y, Nacoulma O, Guissou IP, Guede Guina F. Evaluation *in vivo* et *in vitro* de la toxicité des extraits aqueux d'écorces de tiges et de raciness de *Mitragyna inermis*(Wild). O. Ktz (Rubiaceae). *Pharm Med Trad Afr*, 2001; 11:13-29.
11. Ryan JC. Toward An Ethics of Reciprocity: Ethnobotanical Knowledge and Medicinal Plantsas Cancer Therapies. *Humanities*, 2014; 3:624-644.
12. Philip CC. Nutrition et cancer, Nur Clin Méthabol, 2001; 15:286-297.
13. Létonturier P, Immunologie générale, Masson-Paris, 2001; 53-55.
14. Devulder B, Hatron PY, Hachulla E. Médecine interne. *Abrégé Masson*, 2002.
15. Rajest K, Achyt N, *In vivo* evaluation of anti-oxidant and anti-lipidimic potential of *Annonasquamosal* aqueous extract in type 2 diabetic modes. Journal Ethnopharmacology 2008; 118:21-25.

HOW TO CITE THIS ARTICLE
Jonas MC, Celestine NL, Wilfrid EOA, Robin OP, Maurille OJ, Antoine AA. *In vitro* immunitary impact and antioxidant activity of aqueous extracts of *Maprounea africana* Müll (Euphorbiaceae) and *Mitragyna stipulosa* O.Kze (Rubiaceae). J Phytopharmacol 2017; 6(5):260-263.