Published online: 2020-04-23

Evaluation of Anti-inflammatory, Anti-pyretic, Analgesic, and Hepatoprotective Properties of *Terminalia macroptera*

OPEN ACCESS

Authors
Mahamane Haïdara[1, 2 *], Adama Dénou[2 *], Mohamed Haddad[1], Aïssata Camara[1, 3], Korotoumou Traoré[4], Agnès Aubouy[1], Geneviève Bourdy[1], Rokia Sanogo[2, 5]

Affiliations
1 UMR 152 PHARMA-DEV, Université de Toulouse, IRD, UPS, France
2 Faculté de Pharmacie, Université des Sciences, des Techniques et des Technologies, Bamako, Mali
3 Institute for Research and Development of Medicinal and Food Plants of Guinea (IRDPMAG), Dubréka, Guinea
4 Aidemet ONG, Bamako, Mali
5 Département de Médecine Traditionnelle (DMT), Institut National de Recherche en Santé Publique (INRSP), Bamako, Mali

Key words
Terminalia macroptera, Combretaceae, Médicaments Traditionnels Améliorés/improved traditional medicines, medicinal plant antipyretic, anti-inflammatory, hepatoprotective

received 07.10.2019
revised 18.03.2020
accepted 19.03.2020

Bibliography
DOI https://doi.org/10.1055/a-1142-7072
Planta Med Int Open 2020; 7: e58–e67

ISSN 2509-9264

Correspondence
Dr. Mahamane Haidara
Faculté de Pharmacie
Université des Sciences
des Techniques et des Technologies
BP 1805
Bamako
Mali
Tel.: + 223 76 01 90 68, Fax: + 223 20 29 04 08 / + 223 20 29 04 18
mahamanehaidara83@gmail.com

Dr. Mohamed Haddad
UMR 152 PHARMA-DEV
Université de Toulouse, IRD, UPS
31400 Toulouse
France
Tel.: + 33 5 62 25 98 11, Fax: + 33 5 62 25 98 02
mohamed.haddad@ird.fr

ABSTRACT

In Mali, improved traditional medicines ["Médicaments Traditionnels Améliorés"] are prepared from traditionally used medicinal plants. Recently, the Department of Traditional Medicine has identified *Terminalia macroptera* Guill. & Perr. (Combretaceae) as a potential candidate for an improved traditional medicine. *T. macroptera* is a West African medicinal plant used in Mali against various health disorders, with more than 30 different indications mentioned by traditional healers, including hepatitis, gonorrhea, fever, pain relief, and various infectious diseases (*Helicobacter pylori*-associated diseases). To date, validation of most of the biological activities of has been mainly carried out *in vitro*, except for antimalarial activities. In this study, the potential anti-inflammatory, antipyretic, analgesic, and hepatoprotective properties of *T. macroptera* were investigated in different murine models. Administration of *T. macroptera* ethanolic root and leaf extracts in rats significantly reduced pyrexia, pain, inflammation, and hepatic marker enzymes such as alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase in the different murine models used ($p < 0.05$). A phytochemical screening of *T. macroptera* revealed the presence of tannins, flavonoids, saponins, anthracene derivatives, sterols, triterpenes, and sugars in both leaf and root extracts as the main phytochemical compounds. This was confirmed by qualitative analysis, liquid chromatography coupled with high-resolution mass spectrometry. *T. macroptera* extracts demonstrated interesting *in vivo* antipyretic, analgesic, anti-inflammatory, and hepatoprotective activities. Therefore, *T. macroptera* should be proposed and further evaluated as a potential improved traditional medicine for the treatment of liver-related disorders and for the relief of pain and fever.

* These authors contributed equally to this work.

Introduction

Research on medicinal plants in Mali has been dynamic since political independence in 1960. In 1968, the Department of Traditional Medicine (DMT) was created under the authority of the Ministry of Health. One of the objectives of the DMT is to assess the biological activities of medicinal plants used in traditional medicine, and to formulate and produce phytomedicines based on improved traditional medicines called MTAs "Médicaments Traditionnels Améliorés". MTAs are categorized into four types. The requirements to get an official marketing authorization vary according to the category (▶ **Table 1**). The basic requirement is documenting the traditional uses of the remedy, its safety and efficacy, indicating its standardized dosage, and providing quality control data. Today, the majority of registered MTAs are classified as Category 2, for which clinical trial data is not mandatory [1]. Recently, the DMT identified *Terminalia macroptera* Guill. & Perr. (Combretaceae) as a potential candidate for an MTA.

This plant is widely used in West African countries to treat many health disorders, although research that proves biological activity remains scarce. Eleven publications reported the traditional use of different parts of the plant in the treatment of disorders such as liver disease, malaria, urinary tract infection, diarrhea, pain, fever, and wounds (▶ **Table 2**). *In vitro* antibacterial, antifungal, antiplasmodial, antitrypanosomal, leishmanicidal, and antiviral activities have been previously reported in the roots, leaves, and bark of this species. Reported *in vitro* biological activities include antioxidant, enzyme inhibition, antiproliferative, hemolytic, and immunomodulatory effects (▶ **Table 3**). Only two *in vivo* studies have been carried out [2]. In a recent study, our team demonstrated the ability of *T. macroptera* roots (TMR) and leaves (TML) to limit *Plasmodium* parasitemia and to increase survival in two murine models of uncomplicated and cerebral malaria, respectively. In addition, we demonstrated that according to the Organization for Economic Cooperation and Development's (OECD) Globally Harmonized System of Classification, both extracts were non-toxic orally [3]. The same batch of *T. macroptera* was used in both studies.

In order to complete these data and to investigate some of the ethnopharmacological claims, especially the use of *T. macroptera* as an antipyretic, anti-inflammatory, hepatoprotective, and analgesic agent, our team conducted the first recorded *in vivo* studies to evaluate these properties. The study aims to provide a stronger research basis for the potential future use of *T. macroptera* as an MTA in Mali.

Results and Discussion

At present, the following chemical labelling and classification of acute systemic toxicity, based on oral LD_{50} values, are from the recommendations of the Globally Harmonized System of Classification [OECD, 2008], and ranked as: very toxic, ≤ 5 mg/kg; toxic, > 5 ≤ 50 mg/kg; harmful, > 50 ≤ 500 mg/kg; and no label, > 500 ≤ 2000 mg/kg [4]. In our previous work [3], oral administration of TML and TMR at a dose of 2000 mg/kg did not cause mortality among experimental animals. This indicates that the LD_{50}s of TML and TMR are

▶ **Table 1** MTA classes according to the Ministry of Health Regulation in Mali and requested items for marketing authorization delivery [1].

		MTA class			
		1	2	3	4
	Description	**Traditional medicine prepared by a traditional health practitioner for an individual patient with fresh or dried raw materials, with a short shelf life**	**Traditional medicine currently used in the community, prepared in advance, and composed of crude raw materials**	**Standardized extracts prepared in advance following scientific research**	**Molecules purified from traditional medicines following scientific research**
Requested items for marketing authorization delivery	Covering letter[1]	X	X	X	X
	Samples[2]	X	X	X	X
	Administrative dossier[3]	X	X	X	X
	Pharmaceutical dossier[4]		X	X	
	Expert analytical report[5]		X	X	X
	Pharmacology and toxicology dossier[6]			X	X
	Clinical dossier[7]			X	X
	Expert report on traditional use[8]	X	X	X	X
	Fees[9]	X	X	X	X

[1]Addressed to the Ministry of Health, including the name and address of the manufacturer. [2]Ten samples as sold. [3]Registration form of the manufacturer and memoranda of understanding between the manufacturer and a research institution. [4]Complete monograph(s) of the plant's component(s). Method and stages of preparation and production and Expert report on Good Manufacturing Practices. [5]Quality control method for raw materials. Results of stability and quality control tests of raw materials and excipients. Method and results of quality control during production. Quality control results of the finished product. Stability tests results of the finished product. [6]Pharmacodynamic data. Results of acute and subchronic toxicity tests. Literature review of pharmacology and toxicology. Expert report on the tests carried out. [7]Ethical approval for clinical trials. Clinical trial protocol following standard methods (phase I and II). Results of clinical trials. Expert report on clinical trials carried out. [8]Evidence of the long history of use of the medicine in its current or traditional form (minimum 20 years). Detailed presentation of known toxicological risks. Risks of incorrect use of the medicine. Risks of physical or psychologic dependence. [9]Registration fees receipt.

greater than 2000 mg/kg in albino Swiss mice. Therefore, according to this classification, these fractions can be classed as Category 5 and considered to have relatively low acute toxicity. To work under the most favorable experimental conditions, *T. macroptera* extracts were used in the experiments at 100, 200, and 400 mg/kg for antipyretic, analgesic, anti-inflammatory, and hepatoprotective activity, corresponding to doses 20, 10, and 5 times lower than the safe dose, respectively.

▶ **Table 2** Traditional uses of *T. macroptera* described in different countries of West Africa.

Indications	Number of quotes	Country of quote	Reference
Liver diseases	6	Burkina Faso, Guinea-Bissau, Mali, Senegal	[5, 30–34]
Malaria	5	Burkina Faso, Guinea, Mali, Senegal	[5, 32, 33, 35, 36]
Urinary tract infection	5	Burkina Faso, Guinea-Bissau, Mali, Senegal	[5, 31–34]
Diarrhea	5	Burkina Faso, Mali, Nigeria	[2, 32–34, 37]
Pain	3	Mali, Senegal	[5, 32, 34]
Fever	3	Mali	[5, 30, 34]
Wound	3	Mali, Senegal	[5, 32, 34]
Asthenia	2	Mali, Senegal	[5, 32]
Snake bite	2	Mali, Senegal	[32, 38]
Cough	2	Mali	[34, 37]
Skin diseases and boils	2	Burkina Faso, Mali	[33, 38]
Aphrodisiac	1	Senegal	[32]
Conjunctivitis	1	Senegal	[32]
Gastric ulcer	1	Burkina Faso	[33]
Absence or delay of menstruation	1	Mali	[37]

Leaves and roots of *T. macroptera* are traditionally used for the treatment of inflammatory conditions like pain, fever, and liver diseases [5]. However, according to the literature review, the analgesic, antipyretic, and anti-inflammatory properties of *T. macroptera* have never been investigated before. Other species of *Terminalia* have, however, demonstrated analgesic, antipyretic, and anti-inflammatory properties in studies. The methanolic extract of the leaves of *Terminalia arjuna* (Roxb, ex DC.) Wight & Arn. (100 mg/kg, po) demonstrated *in vivo* analgesic and anti-inflammatory activities with a 51 % inhibition of acetic acid-induced pain and a 75 % inhibition of edema from the third hour of carrageenan injection [6], respectively. Analgesic and antipyretic activities were also shown in the ethanolic extract of the fruit of *Terminalia bellirica* (Gaertn.) Roxb. (200 mg/kg, p.o.) with a 62 % inhibition of acetic acid-induced pain and a reduction of yeast-induced pyrexia from the first hour after administration of the treatment [7].

The effect of ethanolic crude extracts on yeast pyrexia in rats is shown in ▶ **Table 4**. Pyrexia was significantly reduced by TMR and TML treatments when compared to gavage with distilled water. Interestingly, TML at 400 mg/kg was able to lower body temperature 1 h after treatment (T25) ($p < 0.0001$). At the same dose (400 mg/kg), TMR was active against pyrexia 2 h after treatment (T26). Three hours after treatment (T27, T28), the three doses (100, 200, and 400 mg/kg) showed similar efficacy, efficiently reducing pyrexia ($p < 0.05$). Paracetamol (100 mg/kg), which was used as a reference drug, also significantly reduced pyrexia from T25 to T28 when compared to the water-treated group ($p < 0.05$), similarly to TMR and TML from T27 ($p > 0.05$). In addition, TMR and TML ethanolic crude extracts were highly effective in reducing the number of contortions induced by acetic acid compared to distilled water (▶ **Fig. 1a**). Efficacy using the three doses (100, 200, and 400 mg/kg) ($p < 0.0001$ for all) was similar to the efficacy observed after treatment with paracetamol. The maximum analgesic effect was obtained after using 400 mg/kg of TMR, with a pain inhibition of 78.3 ± 0.8 %.

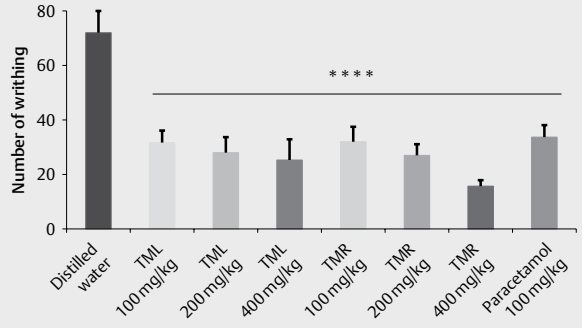
▶ **Table 3** Pharmacological activities of *T. macroptera* reported in the literature.

Plant parts used	Type of test	Pharmacological activities	Model/test used	Reference
Leaf, stem bark, and root bark	*In vitro*	Antibacterial	*In vitro* microdilution, disk diffusion, and direct bioautographic assay	[11, 39–42]
Leaf, stem bark, and root bark	*In vitro*	Antifungal	Disk diffusion assay	[43]
Root bark	*In vitro*	Antiplasmodial	Fluorometric assay	[3, 36, 44]
Root bark	*In vitro*	Antitrypanosomal	Fluorometric assay	[44]
Root bark	*In vitro*	Leishmanicidal	Fluorometric assay	[44]
Root	*In vitro*	Antiviral	*In vitro* antiviral assay by titration	[45]
Leaf, stem bark, and root bark	*In vitro*	Antioxidant	DPPH radical scavenging	[11–13]
Root	*In vitro*	Antiproliferative	Trypan blue assay	[12]
Leaf, stem bark, and root bark	*In vitro*	Enzymatic inhibitor	*In vitro* inhibition of α-glucosidase, 15-lipoxygenase, and xanthine oxidase assay	[13]
Leaf	*In vitro*	Hemolytic	Colorimetric assay	[11]
Leaf, stem bark, and root bark	*In vitro*	Immunomodulatory	Complement fixation assay	[13, 20–21]
Root and leaf	*In vivo*	Antimalarial	Parasitemia and survival evaluation in *P. Plasmodium chabaudi* and *P. Plasmodium bergei ANKA*-infected mice models	[3]
Barks	*In vivo*	Antidiarrheal	Castor oil-induced diarrhea in rats	[11]

▶ **Table 4** Antipyretic activity of *T. macroptera* extracts on yeast-induced pyrexia in Wistar rats.

Group	Temperature (°C)					
	Basal temperature (T0)	T24	T25	T26	T27	T28
Distilled water	37.3 ± 0.4	38.8 ± 0.2	38.9 ± 0.04	38.8 ± 0.1	38.7 ± 0.2	38.6 ± 0.1
TML 100 mg/kg	37.3 ± 0.3	38.2 ± 0.3	39.0 ± 0.3	38.7 ± 0.2	37.7 ± 0.3[d]	37.7 ± 0.1[d]
TML 200 mg/kg	37.0 ± 0.1	38.3 ± 0.3	38.7 ± 0.4	38.5 ± 0.3	37.6 ± 0.2[c]	37.3 ± 0.1[d]
TML 400 mg/kg	37.8 ± 0.3	38.9 ± 0.5	38.2 ± 0.4[b]	38.1 ± 0.3[a]	37.8 ± 0.4[c]	37.7 ± 0.5[c]
TMR 100 mg/kg	37.3 ± 0.3	38.2 ± 0.3	39.0 ± 0.3	38.7 ± 0.2	37.7 ± 0.3[d]	37.7 ± 0.1[d]
TMR 200 mg/kg	37.1 ± 0.2	38.3 ± 0.3	38.8 ± 0.6	38.7 ± 0.4	37.9 ± 0.4[c]	37.4 ± 0.2[d]
TMR 400 mg/kg	37.2 ± 0.3	39.0 ± 0.6	38.7 ± 0.4	37.9 ± 0.6[c]	37.7 ± 0.3[d]	37.7 ± 0.4[c]
Paracetamol 100 mg/kg	37.1 ± 0.1	38.3 ± 0.4	38.1 ± 0.5[b]	37.6 ± 0.3[d]	37.2 ± 0.2[d]	37.2 ± 0.2[d]

The basal rectal temperature of the rats was taken using a digital clinical thermometer (T0). At the end of the day (T8), each animal was given a subcutaneous injection of a 20 % w/v aqueous suspension of yeast and then fasted overnight. The rectal temperature of the animals was taken 16 h after the yeast injection (T24). The rectal temperature of the rats was taken hourly during the 4 h following the administration of the treatments (T25 to T28). Data are expressed as the mean ± SD (n = 6 per group). Two-way ANOVA followed by Dunnett's multiple comparison tests were used for analysis. Statistical significance $p < 0.05$. [a]$P < 0.05$ compared to the distilled water group, [b]$p < 0.01$ compared to the distilled water group, [c]$p < 0.001$ compared to the distilled water group, [d]$p < 0.0001$ compared to the distilled water group.



▶ **Fig. 1** Analgesic activity of T. macroptera leaf (TML) and root (TMR) extracts on acetic acid-induced writhing in Swiss mice. One-way ANOVA followed by Dunnett's multiple comparison tests were used for analysis. Treatment efficacies were compared to distilled water; **** $p < 0.0001$.

Furthermore, TMR and TML ethanolic crude extracts at the three doses (100, 200, and 400 mg/kg) were able to significantly reduce edema 3 h after carrageenan injection when compared to the distilled water treatment. Indomethacin, the reference treatment, presented similar efficacy. The maximum anti-inflammatory effect (92.6 ± 7.6 %) was obtained with TML treatment at 400 mg/kg 5 h after carrageenan injection (▶ **Table 5**).

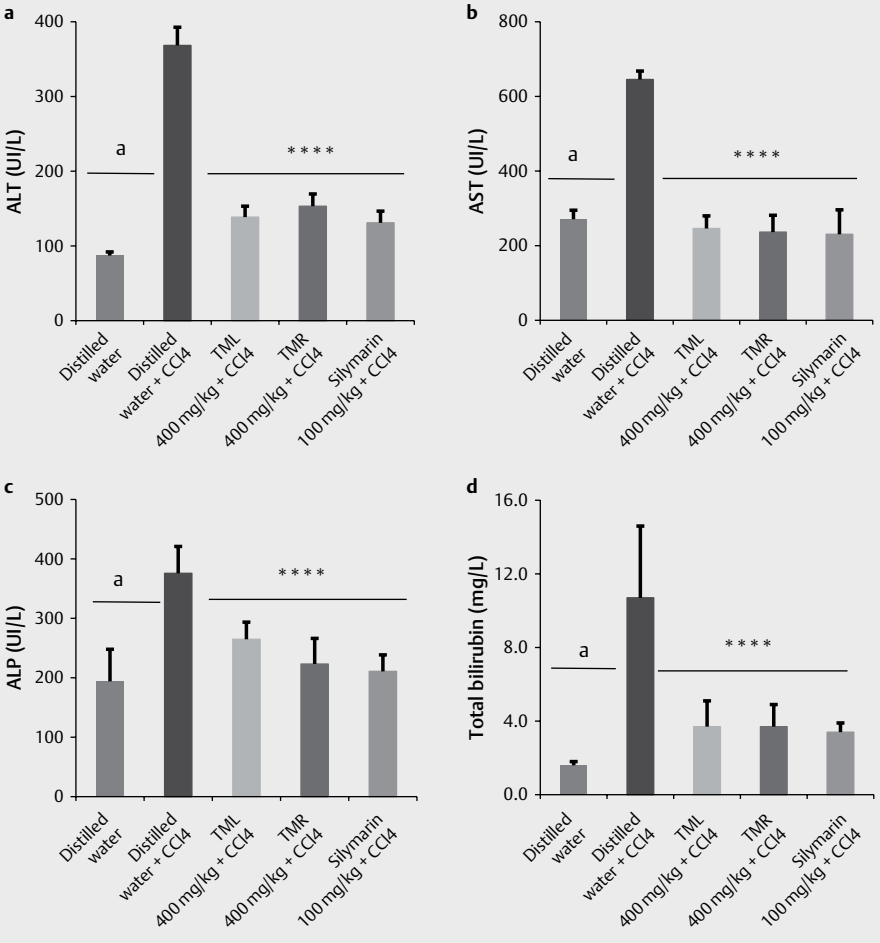
Fever, pain, and inflammation are clinical manifestations associated with a wide range of diseases. Fever is one of the symptoms marking the onset of an infection or inflammation. The use of *T. macroptera* in feverish illnesses might be explained by its ability to interfere with the fever pathway, where exogenous pyrogens, such as microbes and/or their toxins, stimulate mononuclear phagocytes to release proinflammatory and pyrogenic cytokines such as TNF-α, IL1, IL-6, and IFNγ[8]. The release of such cytokines was also shown to be associated with pain in the murine model we used to measure the analgesic activity of *T. macroptera*, based on acetic acid-induced writhing [9]. These pyrogenic cytokines trigger arachidonic acid metabolism, including prostaglandin E2 (PGE2) production, a lipid mediator largely associated with fever and inflammation [8]. Furthermore, in the present study, we screened the efficacy of extracts of *T. macroptera* in a mouse model of CCl_4-induced acute hepatic damage to test its potential as a potent hepatoprotective medicinal plant. CCl_4 administration induces a high hepatocyte injury, leading to the extensive formation of free radicals such as trichloromethyl and peroxytrichloromethyl, which are highly toxic for the liver [10]. The effect of TMR and TML treatments at 400 mg/kg on hepatic marker enzymes in CCl_4-induced hepatic injury in rats is shown in ▶ **Fig. 2**. The levels of alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), and total bilirubin (TB) were significantly increased in the rat group intoxicated with the intraperitoneal injection of CCl_4, and treated with distilled water (CCl_4 model control) compared with the non-intoxicated rat group treated with distilled water (healthy control) ($p < 0.0001$). TMR and TML treatments significantly reduced the levels of ALT (▶ **Fig. 2a**), AST (▶ **Fig. 2b**), ALP (▶ **Fig. 2c**), and TB (▶ **Fig. 2d**) compared to distilled water in the CCl_4 model controls ($p < 0.0001$) (▶ **Fig. 2**). Several studies have demonstrated the antiradical *in vitro* activity of leaf and root extracts of *T. macroptera*11–13], suggesting that the hepatoprotective activity of our extracts could partly be due to the inhibition of the production of these free radicals. Furthermore, TMR and TML efficacy was similar to that of the reference drug silymarin. Interestingly, levels of hepatic enzymes obtained after TML, TMR, and silymarin treatments were similar to the healthy control group. These results are consistent with those from studies carried out on other species of *Terminalia*. These also demonstrated dose-dependent hepatoprotective properties by reducing transaminases and alkaline phosphatase such as *T. bellirica* fruits [14] and *Terminalia catappa* leaves [15].

In this study, a qualitative analysis by LC-HRMS of *T. macroptera* extracts was undertaken to compare their composition and biological activities. A total of 59 compounds were detected and identified through HRMS and MS/MS fragmentation patterns using MS-FINDER and the DNP database (▶ **Table 6**). The MS-FINDER dereplication method allowed us to annotate the corresponding peaks, mostly found in the Combretaceae family (▶ **Table 3**). We separat-

▶ **Table 5** Anti-inflammatory activity of *T. macroptera* extracts on carrageenan-induced edema in Swiss mice.

Group	Thickness of paw (mm)				% Inhibition		
	0 h	1 h	3 h	5 h	1 h	3 h	5 h
Distilled water	1.12 ± 0.02	2.04 ± 0.15	2.14 ± 0.30	1.90 ± 0.21			
TML 100 mg/kg	1.13 ± 0.05	1.99 ± 0.22	1.85 ± 0.22[b]	1.62 ± 0.29[b]	11.70 ± 3.90	33.00 ± 12.20	53.30 ± 4.90
TML 200 mg/kg	1.14 ± 0.09	1.96 ± 0.18	1.69 ± 0.17[d]	1.55 ± 0.12[c]	15.80 ± 7.90	45.30 ± 2.90	48.40 ± 9.10
TML 400 mg/kg	1.20 ± 0.09	1.55 ± 0.26[d]	1.42 ± 0.05[d]	1.30 ± 0.06[d]	72.50 ± 15.90	82.40 ± 1.10	92.60 ± 7.60
TMR 100 mg/kg	1.19 ± 0.08	1.95 ± 0.08	1.84 ± 0.10[b]	1.50 ± 0.12[d]	17.40 ± 3.30	32.50 ± 18.70	63.50 ± 17.80
TMR 200 mg/kg	1.15 ± 0.04	1.80 ± 0.11[a]	1.68 ± 0.10[d]	1.47 ± 0.07[d]	28.60 ± 3.70	46.40 ± 9.00	59.10 ± 0.80
TMR 400 mg/kg	1.13 ± 0.04	1.50 ± 0.06[d]	1.38 ± 0.08[d]	1.32 ± 0.04[d]	60.70 ± 1.80	75.10 ± 0.50	78.50 ± 5.10
Indomethacin 8mg/kg	1.14 ± 0.04	1.88 ± 0.06	1.73 ± 0.24[d]	1.45 ± 0.13[d]	19.60 ± 8.60	55.60 ± 5.10	66.30 ± 2.90

Data are expressed as the mean ± SD (n = 6 per group). Two-way ANOVA followed by Dunnett's multiple comparison tests were used for analysis. Statistical significance $p < 0.05$. [a]$P < 0.05$ compared to the distilled water group, [b]$p < 0.01$ compared to the distilled water group, [c]$p < 0.001$ compared to the distilled water group, [d]$p < 0.0001$ compared to the distilled water group.



▶ **Fig. 2** Hepatoprotective effects of *T. macroptera* extracts in CCl_4-intoxicated rats. Rats were pretreated with distilled water, TMR, TML, or silymarin daily for 7 days (n = 5 per group). Except for the healthy control group (white sticks), rats were intoxicated with CCl_4 (0.5 mL/kg i.p.) 1 h after the last treatment. **a** Serum levels of ALT in UI/L. **b** Serum levels of AST in UI/L. **c** Serum levels of ALP in UI/L. **d** Total bilirubin in mg/L. Data are expressed as the mean ± SD. One-way ANOVA followed by Dunnett's multiple comparison tests were used for analysis. Statistical significance $p < 0.05$. [a]$P < 0.0001$ compared to the distilled water group; **** $p < 0.0001$ compared to the CCl_4-treated group

ed the detected compounds into three categories: compounds detected only in the roots, compounds detected only in the leaves, and compounds detected in both roots and leaves.

Terminalia species have been shown to contain various secondary metabolites including cyclic triterpenes and their derivatives (flavonoids, tannins, and phenolic acids). In our study, the LC/MS

▶ **Table 6** Putative identified features in roots and leaves (*m/z*×RT pairs) using HRMS and MS/MS fragmentation patterns using ▶ **MS-finder** and the DNP database.

Detected only in the roots			
m/z	**Rt**	**Molecular formula**	**Identity**
681.3842 $[M+H]^+$	3.06	$C_{36}H_{56}O_{12}$	Termiarjunoside II
600.9909 $[M-H]^-$	1.00	$C_{28}H_{10}O_{16}$	Terminalin
521.3469 $[M+H]^+$	3.06	$C_{30}H_{48}O_7$	Bellericagenin B
315.0852 $[M+H]^+$	3.25	$C_{17}H_{14}O_6$	Combretastatin C1
471.3475 $[M+H]^+$	3.62	$C_{30}H_{46}O_4$	2-*α*-Hydroxymicromeric acid
649.3951 $[M+H]^+$	3.36	$C_{36}H_{56}O_{10}$	Quadranoside VIII
991.5133 $[M+H]^+$	3.36	$C_{48}H_{78}O_{21}$	2,3,19,23-Tetrahydroxy-12-oleanen-28-oic acid-(2-*α*,3*β*,19*β*)- 3-O-[*β*-D-Galactopyranosyl-(1->3)-*β*-D-glucopyranoside], 28-O-*β*-D-glucopyranosyl ester
461.0721 $[M-H]^-$	2.52	$C_{21}H_{18}O_{12}$	Ellagic acid-2,8-Di-Me ether, 3-O-*β*-D-xylopyranoside
463.3053 $[M+H]^+$	2.59	$C_{27}H_{42}O_6$	Norquadrangularic acid A
495.0784 $[M-H]^-$	0.59	$C_{21}H_{20}O_{14}$	1,5-Digalloylquinic acid
811.4467 $[M-H]^-$	4.26	$C_{42}H_{68}O_{15}$	Arjunolitin
469.0059 $[M-H]^-$	0.98	$C_{21}H_{10}O_{13}$	Flavogallonic acid
Detected only in the leaves			
m/z	**Rt**		**Identity**
583.1117 $[M-H]^-$	2.64	$C_{28}H_{24}O_{14}$	2''-O-Galloylvitexin
611.1603 $[M+H]^+$	2.32	$C_{27}H_{30}O_{16}$	Quercetin 3-(4-galactosylrhamnoside)
953.0919 $[M-H]^-$	1.00	$C_{41}H_{30}O_{27}$	Terchebin
635.2003 $[M-H]^-$	3.38	$C_{23}H_{40}O_{20}$	*β*-D-Galactopyranosyl-(1->6)-*β*-D-galactopyranosyl-(1->6)-*β*-D-galactopyranosyl-(1->3)-L-arabinose
277.0343 $[M+H]^+$	0.99	$C_{13}H_8O_7$	3,4,8,9,10-Pentahydroxy-6H-dibenzo[b,d]pyran-6-one
321.0240 $[M+H]^+$	1.01	$C_{14}H_8O_9$	Luteolic acid
765.0982 $[M-H]^-$	2.36	$C_{35}H_{26}O_{20}$	Ellagic acid-3-Me ether, 7-O-[3,4,5-trihydroxybenzoyl-(->3)-[3,4,5-trihydroxy-benzoyl-(->4)]-*α*-L-rhamnopyranoside]
699.3561 $[M+H]^+$	4.74	$C_{35}H_{54}O_{14}$	3,14,19-Trihydroxycard-20(22)-enolide-(3*β*,5*β*,14*β*)-form-3-O-[*β*-D-galactopyranosyl-(1->4)-*α*-L-rhamnopyranoside]
473.1484 $[M-H]^-$	0.37	$C_{17}H_{30}O_{15}$	*β*-D-Galactopyranosyl-(1->6)-*β*-D-galactopyranosyl-(1->3)-L-arabinose
895.0855 $[M-H]^-$	2.74	$C_{39}H_{28}O_{25}$	Quisqualin A
295.1024 $[M-H]^-$	0.42	$C_{18}H_{16}O_4$	Combretastatin D2
Detected in both the leaves and roots			
m/z	**Rt**		**Identity**
955.1099 $[M-H]^-$	2.50	$C_{41}H_{32}O_{27}$	Chebulinic acid
635.0901 $[M-H]^-$	1.15	$C_{27}H_{24}O_{18}$	1,3,6-Trigalloylglucose-*β*-D-Pyranose
785.0877 $[M-H]^-$	2.24	$C_{34}H_{26}O_{22}$	Tercatain
609.1478 $[M-H]^-$	2.27	$C_{27}H_{30}O_{16}$	Quercetin 3-(4-galactosylrhamnoside)
331.0668 $[M-H]^-$	0.39	$C_{13}H_{16}O_{10}$	3-Galloylglucose
503.3368 $[M-H]^-$	4.07	$C_{30}H_{48}O_6$	Belleric acid
197.0460 $[M-H]^-$	1.33	$C_9H_{10}O_5$	4-O-Ethylgallic acid
633.0734 $[M-H]^-$	0.68	$C_{27}H_{22}O_{18}$	Corilagin
801.4097 $[M-H]^-$	3.54	$C_{43}H_{62}O_{14}$	2,3,23-Trihydroxy-12-oleanen-28-oic acid-(2*α*,3*β*)-23-O-(3,4,5-Trihydroxybenzoyl), 28-O-*β*-D-glucopyranosyl ester
447.0576 $[M-H]^-$	2.04	$C_{20}H_{16}O_{12}$	Eschweilenol C
631.3835 $[M-H]^-$	4.11	$C_{36}H_{56}O_9$	Jessic acid-3-O-*α*-L-arabinopyranoside
793.4412 $[M-H]^-$	3.50	$C_{42}H_{66}O_{14}$	2,3,19-Trihydroxy-12-oleanen-28-oic acid-(2*α*,3*α*,19*α*)-3-Ketone, 28-O-[*α*-L-rhamnopyranosyl-(1->4)-*β*-D-glucopyranosyl] ester
451.3208 $[M+H]^+$	3.37	$C_{30}H_{42}O_3$	Erythrophyllic acid
1083.0579 $[M-H]^-$	0.34	$C_{48}H_{28}O_{30}$	Isoterchebulin
639.3526 $[M-H]^-$	4.16	$C_{37}H_{52}O_9$	23-Galloylarjunolic acid
933.0627 $[M-H]^-$	0.41	$C_{41}H_{26}O_{26}$	Arjunin
781.0525 $[M-H]^-$	0.40	$C_{34}H_{22}O_{22}$	Isoterchebuloylglucose

▶ **Table 6** Continued

Detected in both the leaves and roots			
m/z	**Rt**		**Identity**
359.1492 $[M+H]^+$	3.70	$C_{20}H_{22}O_6$	2',4',5,7-Tetrahydroxy-8-methylflavanone-Tetra-Me ether. 2',4',5,7-Tetramethoxy-8-methylflavanone
817.4045 $[M-H]^-$	3.32	$C_{43}H_{62}O_{15}$	2,3,6,23-Tetrahydroxy-12-oleanen-28-oic acid-(2*α*,3*β*,6*β*)-23-O-(3,4,5-Trihydroxybenzoyl), 28-O-*β*-D-glucopyranosyl ester
297.1523 $[M-H]^-$	4.65	$C_{19}H_{22}O_3$	4-Hydroxy-4'-methoxy-7,7'-epoxylignan
603.3892 $[M-H]^-$	5.75	$C_{35}H_{56}O_8$	1,3-Dihydroxycycloart-24-en-28-oic acid-(1*α*,3*β*)-3-O-*α*-L-arabinopyranoside
521.0953 $[M-H]^-$	3.27	$C_{23}H_{22}O_{14}$	Flavellagic acid-2,3,8-Tri-Me ether, 7-O-*β*-D-glucopyranoside
783.0685 $[M-H]^-$	0.62	$C_{34}H_{24}O_{22}$	Terflavin B
1085.0729 $[M-H]^-$	0.62	$C_{48}H_{30}O_{30}$	Terflavin A
487.3422 $[M-H]^-$	4.22	$C_{30}H_{48}O_5$	2,6-Dihydroxybetulic acid
667.4056 $[M+H]^+$	3.37	$C_{36}H_{58}O_{11}$	Chebuloside II
329.0313 $[M-H]^-$	3.23	$C_{16}H_{10}O_8$	3,8-Di-O-methylellagic acid
973.1208 $[M-H]^-$	2.43	$C_{41}H_{34}O_{28}$	Neochebulinic acid
311.1681 $[M-H]^-$	4.94	$C_{20}H_{24}O_3$	4,4'-Dimethoxy-7,7'-epoxylignan
485.3257 $[M-H]^-$	4.30	$C_{30}H_{46}O_5$	Lonchoterpene
631.0577 $[M-H]^-$	0.57	$C_{27}H_{20}O_{18}$	Terflavin D
501.3215 $[M-H]^-$	3.90	$C_{30}H_{46}O_6$	Ivorengenin A
513.3162 $[M-H]^-$	3.82	$C_{31}H_{46}O_6$	Methyl quadrangularate N
491.0825 [M - H]-	2.39	$C_{22}H_{20}O_{13}$	Ellagic acid-3,8-Di-Me ether, 2-O-*β*-D-glucopyranoside
681.3880 [M - H]-	3.91	$C_{36}H_{58}O_{12}$	Bellericaside B
505.3543 [M - H]-	3.87	$C_{30}H_{50}O_6$	Quadrangularic acid L

analyses allowed us to highlight the presence of tannins and triterpenes. Phenolic compounds are widely described in the literature for their potential biological activity such as anti-inflammatory and immunomodulatory effects [15, 16]. They are excellent antioxidants due to the presence of a hydroxyl group capable of capturing oxygen free radicals [17]. Of all the identified compounds, only the anti-inflammatory, analgesic, antipyretic, and hepatoprotective properties of ellagic acid have been reported in the literature. Ellagic acid administered orally at doses of 1 to 100 mg/kg in mice presented analgesic, antipyretic, and anti-inflammatory activities [18]. At a dose of 50-100 mg/kg, ellagic acid had hepatoprotective effects [19]. Furthermore, eschweilenol C has also been reported for anti-inflammatory activity in aqueous extracts of *Terminalia fagifolia* by inhibition of the NF*κ*B pathway in lipopolysaccharide-activated microglial cells [16]. These results suggest that the analgesic, antipyretic, anti-inflammatory, and hepatoprotective activities of the ethanolic extract of *T. macroptera* leaves and roots may be due, at least partly, to the presence of ellagic acid and its derivatives.

In addition, we also highlighted the presence of sugars by performing tube staining reactions and by using LC-HRMS analysis, especially polysaccharides. Some of these compounds were previously isolated from the *T. macroptera* leaves and roots harvested in Mali by Zou and colleagues and showed immunomodulatory properties through the complement fixation assay [20, 21]. Polysaccharides isolated from two other plants, *Ganoderma lucidum* and *Panax ginseng*, were shown to have anti-inflammatory and hepatoprotective effects, respectively [22, 23]. These data suggest that the anti-inflammatory activity and hepatoprotective effect of *T. macroptera* may be also due to the presence of polysaccharides, although this remains to be demonstrated. Finally, our study did not reveal the presence of alkaloids, contrary to a previous study [24], whose plant was collected in Nigeria.

Therefore, it would be of great interest to repeat plant collection in different areas of West Africa and at different times of the year in order to verify if alkaloid content depends on environmental conditions. Additionally, it would be valuable to proceed to a more detailed phytochemical analysis of this plant species through the metabolomic approach described in a previous work [25]. This type of dereplication approach would facilitate a better understanding of the link between molecular content and biological properties. Further bioassay-guided fractionation is necessary to confirm the origin of these biological activities, including synergistic potential between tannins, lignans, and terpenoids found in this plant.

In summary, in this study, we have demonstrated, for the first time, the *in vivo* antipyretic, analgesic, anti-inflammatory, and hepatoprotective activities of ethanolic extracts of TML and TMR coupled with a deep phytochemical analysis through metabolomics. These *in vivo* pharmacological effects combined with other *in vitro* activities previously demonstrated in other works suggest that this species may be beneficial in alleviating pathologies associated with symptoms of fever, pain, and inflammation. The hepatoprotective activity of *T. macroptera* could also be useful to treat many different health conditions related to liver integrity, i.e., hepatitis, either viral or toxic. Additionally, it has been previously shown that according to the OECD's Globally Harmonized System of Classification, these extracts can be classified as Category 5 and their

oral administration considered weakly toxic orally. These results are in accordance with the present study since no highly toxic compounds were detected by LC-HRMS of root and leaf extracts. For all these reasons, we propose submitting a request in Mali for authorization of a Category 2 MTA formulation of this species. This MTA should be recommended in cases of hepatitis and liver-related disorders, fever, and pain.

Materials and Methods

Plant material

The leaves and roots of *T. macroptera* were collected in August 2015 in Siby, a village located in the Koulikoro region in Mali. A specimen of the plant, voucher number 3752/DMT, was deposited in the herbarium of the DMT/NIRPH and authenticated by Mr. Seydou Dembele, a forestry engineer. Access and benefit sharing to biodiversity and its associated traditional knowledge was established according to Malian national rules.

Preparation of extracts

T. macroptera leaves and roots were dried under shade at room temperature for 2 weeks and ground into powder before extraction. In Mali, the difficulties linked to drying aqueous extracts led us to choose a polar solvent that can extract the maximum from chemical constituents, and which is easily evaporable on a rotary evaporator. Therefore, we chose 90 % ethanol instead of water, which is usually used as the traditional extraction solvent for technical reasons.

A total of 250 g dried samples was macerated in 1000 mL of 90 % ethanol for 24 h and filtered using Whatman filters N °1. This operation was repeated three times. The three filtrates were combined and evaporated under *vacuo* to dryness (Büchi rotary evaporator Model R-200). Yields of leaf and root extraction were 17.6 % (44 g) and 14 % (35 g), respectively. The crude extracts of *T. macroptera* leaves (TML) and roots (TMR) were stored in a refrigerator at 4–8 °C before use.

Drugs and chemicals

Paracetamol (solid, ≥ 97 %, UC448; Sigma-Aldrich), indomethacin (I8280, ≥ 98.5 %; Sigma-Aldrich), silymarin (mixture of anti-hepatotoxic flavonolignans from the fruit of *Silybum marianum*, S0292; Sigma-Aldrich,), carrageenan (C1138; Sigma-Aldrich), yeast (YBD; Sigma Aldrich), acetic acid (extra pure; Fisher Chemicals), and carbon tetrachloride (99 %; Fisher Chemicals) were used in the pharmacological studies.

Animals and ethics statement

Swiss albino mice (aged 4–6 weeks and weighing 20–25 g) and Wistar rats (aged 8–10 weeks and weighing 100–150 g) of either sex were taken from the DMT/NIRPH animal house. The animals were maintained in standard laboratory conditions (25 °C and light/dark cycles, i.e., 12/12 h) and fed with standard food and tap water. Animal welfare requirements were strictly considered during these experiments, as required by the National Institute for Public Health Research (INRSP) Ethics Committee in Bamako, Mali. INRSP Ethics Committee authorization and approval were obtained (24/2016/CE-INRSP).

Assessment of antipyretic activity

The antipyretic activity of TML and TMR crude ethanolic extracts was assessed using yeast-induced pyrexia in male Wistar rats (100-150 g) [26]. The basal rectal temperature of the rats was taken using a digital clinical thermometer (T0). At the end of the day (T8), each animal was given a subcutaneous injection of a 20 % w/v aqueous suspension of yeast and then fasted overnight. The rectal temperature of the animals was taken 16 h after the yeast injection (T24). The animals with a temperature difference of 0.5 °C were selected, then gathered into eight groups of five rats and submitted to oral gavage. The first group was administered paracetamol (100 mg/kg) as the reference drug. The second group, the control group, was given distilled water (10 mL/kg). The remaining groups were fed with TML and TMR crude extract (100, 200, and 400 mg/kg). The rectal temperature of the rats was taken hourly during the 4 h following the administration of the treatments (T25–T28). The mean value for each group was calculated and compared with the control and reference groups at each time point.

Assessment of analgesic activity

Analgesic activity was tested using Swiss albino mice (20-25 g) of either sex. Animals were randomized into eight groups of six mice (three males and three females). Group I (control group) was administered distilled water orally (25 mL/kg), and Group II (reference drug) was administered paracetamol orally (100 mg/kg). The remaining groups were treated orally with TML and TMR crude ethanolic extracts (100, 200, and 400 mg/kg). One hour after these treatments, the animals were treated by intraperitoneal injection (i.p.) with 1 % acetic acid. The number of abdominal constrictions (writhings) were counted for 20 min, starting 5 min after acetic acid injection [27]. The mean number of writhings for each group was calculated and compared with that of the control and reference groups. The percentage of inhibition was calculated using the following formula:

$$\%\,\text{Inhibition} = \frac{(Wc - Wt) \times 100}{Wc}$$

Where Wt means the number of writhings in the test animals and Wc means the number of writhings in the controls.

Assessment of anti-inflammatory activity

Acute inflammation was produced by an injection of carrageenan (an edematogenic agent) into the subplantar region of the right hind paw of the mice [28]. Swiss albino mice (20–25 g) of either sex were randomized into eight groups of six mice (three males and three females). Groups I and II were treated orally with distilled water (25 mL/kg) as the control group and with indomethacin (8 mg/kg) as the reference drug, respectively. The remaining groups were treated orally with TML and TMR crude ethanolic extracts (100, 200, and 400 mg/kg). One hour after the treatments (T0), 0.025 mL of 1 % carrageenan suspension was subcutaneously injected into the right hind paw of the animals. Paw thickness was measured using a sliding caliper before injection (V0) and after 1, 3, and 5 h (VT). The edema volume was estimated by subtracting the value of V0 to VT (1, 3, and 5 h after the injection). The average paw thickness of each group of mice was calculated and compared

with that of the control and reference groups. The percentage of inhibition was calculated using the following formula:

$$\%\,\text{Inhibition} = \frac{([(VT - V0)\text{control} - (VT - V0)\text{treated group}] \times 100)}{(VT - V0)\text{control}}$$

Assessment of hepatoprotective activity

The hepatoprotective activity of the extracts was assessed using an intraperitoneal injection of carbon tetrachloride (CCl_4) in male Wistar rats using the method described in a previous work [29]. The insufficient number of rats at the time of the test led us to evaluate one dose (400 mg/kg) of each extract.

The rats (100–150 g) were randomized into five groups of five rats each and treated once a day for 7 days. Groups I and II received distilled water (10 mL/kg, orally) as the control groups, and Group III received silymarin (100 mg/kg, orally) as the reference hepatoprotective drug. Groups IV and V received crude ethanolic extracts of TML and TMR (400 mg/kg, orally). One hour after treatment on day 7, the rats of groups II–V were intoxicated with an intraperitoneal administration of 0.5 mL/kg CCl_4 (1:1 in olive oil). Twenty-four hours after oral administration of the hepatotoxic agent, rats were anesthetized with ether, blood was collected from the retro-orbital plexus, and the serum was separated by centrifugation at 2500 rpm. ALT, AST, ALP, and TB were measured in the serums using a BS200 MINDRAY biochemistry automaton. The values obtained were compared between treatment groups.

Metabolites profiling by UHPLC-HRMS

Metabolite profiles of the TMR ethanol extract (1 mg/mL) were acquired using a UHPLC-DAD-CAD-LTQ Orbitrap XL instrument (Thermo Fisher Scientific) equipped with an electrospray ionization (ESI) source. The UHPLC system consisted of an Ultimate 3000 UHPLC (Thermo Fisher Scientific) equipped with an Acquity BEH C18 column (100 × 2.1 mm i.d., 1.7 µm; Waters). The mobile phase was composed of solvent A (0.1 % formic acid-water) and solvent B (0.1 % formic acid-acetonitrile) with a gradient elution (0-0.5 min, 95 % A; 0.5-12 min, 95-5 % A; 12-15 min, 5 % A; 15-15.5 min, 5-95 % A; 15.5-19 min, 95 % A). The flow rate of the mobile phase was 0.45 mL/min. The injection volume was 4 µL and the column temperature was maintained at 40 °C. ESI was applied in negative ion (NI) and positive ion (PI) mode under the following conditions: capillary voltage at 3.0 and 4.2 kV for NI and PI, respectively, and capillary temperature at 300 °C. The UV detection was performed by a diode array detector from 210 to 400 nm. Full mass spectra were recorded between 100 and 1500 Da. Collision-induced dissociation mass spectra were obtained using the following parameters: 35 % normalized collision energy, isolation width 2 Da, activation Q 0.250. External mass calibration was accomplished before starting the experiment [25].

Statistical analysis

The results are expressed as the mean ± SEM. The data was analyzed using GraphPad Prism 6 Software. Statistical analysis was performed by ANOVA (one-way for analgesic and hepatoprotective activity and two-way for antipyretic and anti-inflammatory activity), followed by Dunnett's test. The differences were considered significant if the p value was less than 0.05.

Acknowledgments

The authors are grateful to the DMT technical team and partners for their technical support. This publication was made possible thanks to the support provided by the "Direction des programmes de recherche et de la formation au Sud" of the French Institute of Research for Development (IRD-DPF) and by the Training Program for Trainers (PFF) in Mali. Special thanks to Elizabeth Elliott and Marieke Audureau for proofreading this article.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Willcox M, Sanogo R, Diakite C, Giani S, Paulsen BS, Diallo D. Improved traditional medicines in Mali. J Altern Complement Med 2012; 18: 212–220

[2] Etuk EU, Ugwah MO, Ajagbonna OP, Onyeyili PA. Ethnobotanical survey and preliminary evaluation of medicinal plants with antidiarrhea properties in Sokoto state. Nigeria. J Med Plants Res 2009; 3: 763–766

[3] Haidara M, Haddad M, Denou A, Marti G, Bourgeade-Delmas S, Sanogo R, Bourdy G, Aubouy A. In vivo validation of anti-malarial activity of crude extracts of Terminalia macroptera, a Malian medicinal plant. Malar J 2018; 17: 68–77

[4] Organization for Economic Co-operation and Development. OECD guidelines for the testing of chemicals Section 4: Health effects. Test no 425: acute oral toxicity: Up-and-down procedure. Paris: OECD Publishing; 2008

[5] Malgras D. Arbres et arbustes guérisseurs des savanes maliennes. Editions KARTHALA et ACCT. Paris: KARTHALA et ACCT; 1992

[6] Biswas M, Biswas K, Karan TK, Bhattacharya S, Ghosh AK, Haldar PK. Evaluation of analgesic and anti-inflammatory activities of Terminalia arjuna leaf. J Phytol 2011; 3: 33–38

[7] Sharma US, Sharma UK, Singh A. Screening of Terminalia bellirica fruits extracts for its analgesic and antipyretic activities. Jordan J Biol Sci 2010; 3: 121–124

[8] Prajitha N, Athira S, Mohanan P. Pyrogens a polypeptide produces fever by metabolic changes in hypothalamus: mechanisms and detections. Immunol Lett 2018; 204: 38–46

[9] Ribeiro RA, Vale ML, Thomazzi SM, Paschoalato AB, Poole S, Ferreira SH, Cunha FQ. Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. Eur J Pharmacol 2000; 387: 111–118

[10] Clawson GA. Mechanisms of carbon tetrachloride hepatotoxicity. Pathol Immunopathol Res 1989; 8: 104–112

[11] Karou SD, Tchacondo T, Ouattara L, Anani K, Savadogo A, Agbonon A, Attaia MB, de Souza C, Sakly M, Simpore J. Antimicrobial antiplasmodial haemolytic and antioxidant activities of crude extracts from three selected Togolese medicinal plants. Asian Pac J Trop Med 2011; 4: 808–813

[12] Tagne RS, Telefo BP, Nyemb JN, Yemele DM, Njina SN, Goka SMC, Lienou LL, Kamdje AHN, Moundipa PF, Farooq AD. Anticancer and antioxidant activities of methanol extracts and fractions of some Cameroonian medicinal plants. Asian Pac J Trop Med 2014; 7: S442–S447

[13] Zou YF, Ho GTT, Malterud KE, Le NHT, Inngjerdingen KT, Barsett H, Diallo D, Michaelsen TE, Paulsen BS. Enzyme inhibition antioxidant and immunomodulatory activities and brine shrimp toxicity of extracts from the root bark stem bark and leaves of Terminalia macroptera. J Ethnopharmacol 2014; 155: 1219–1226

[14] Jadon A, Bhadauria M, Shukla S. Protective effect of Terminalia bellerica Roxb. and gallic acid against carbon tetrachloride induced damage in albino rats. J Ethnopharmacol 2007; 109: 214–218

[15] Abiodun OO, Rodríguez-Nogales A, Algieri F, Gomez-Caravaca AM, Segura-Carretero A, Utrilla MP, Rodriguez-Cabezas ME, Galvez J. Antiinflammatory and immunomodulatory activity of an ethanolic extract from the stem bark of Terminalia catappa L. (Combretaceae): in vitro and in vivo evidences. J Ethnopharmacol 2016; 192: 309–319

[16] Rodrigues de Araújo A, Iles B, de Melo Nogueira K, Dias J, do N, Plácido A, Rodrigues A, Albuquerque P, Silva-Pereira I, Socodatto R, Portugal CC, Relvas JB, Costa Véras LM, Dalmatti Alves Lima FC, Batagin-Neto A, Rolim Medeiros JV, Moreira Nunes PH, Eaton P, de Souza de Almeida Leite JR. Antifungal and anti-inflammatory potential of eschweilenol C-rich fraction derived from Terminalia fagifolia Mart. J Ethnopharmacol 2019; 240: 111941

[17] Manosroi A, Jantrawut P, Ogihara E, Yamamoto A, Fukatsu M, Yasukawa K, Tokuda H, Suzuki N, Manosroi J, Akihisa T. Biological activities of phenolic compounds and triterpenoids from the galls of Terminalia chebula. Chem Biodivers 2013; 10: 1448–1463

[18] Rogerio AP, Fontanari C, Melo MCC, Ambrosio SR, de Souza GEP, Pereira PS, França SC, da Costa FB, Albuquerque DA, Faccioli LH. Anti-inflammatory analgesic and anti-oedematous effects of Lafoensia pacari extract and ellagic acid. J Pharm Pharmacol 2006; 58: 1265–1273

[19] Girish C, Pradhan SC. Hepatoprotective activities of picroliv curcumin and ellagic acid compared to silymarin on carbon-tetrachloride-induced liver toxicity in mice. J Pharmacol Pharmacother 2012; 3: 149–155

[20] Zou YF, Zhang BZ, Inngjerdingen KT, Barsett H, Diallo D, Michaelsen TE, Paulsen BS. Complement activity of polysaccharides from three different plant parts of Terminalia macroptera extracted as healers do. J Ethnopharmacol 2014; 155: 672–678

[21] Zou YF, Barsett H, Ho GTT, Inngjerdingen KT, Diallo D, Michaelsen TE, Paulsen BS. Immunomodulating pectins from root bark stem bark and leaves of the Malian medicinal tree Terminalia macroptera structure activity relations. Carbohydr Res 2015; 403: 167–173

[22] Joseph S, Sabulal B, George V, Antony K, Janardhanan K. Antitumor and anti-inflammatory activities of polysaccharides isolated from Ganoderma lucidum. Acta Pharmaceutica 2011; 61: 335–342

[23] Shim JY, Kim MH, Kim HD, Ahn JY, Yun YS, Song JY. Protective action of the immunomodulator ginsan against carbon tetrachloride-induced liver injury via control of oxidative stress and the inflammatory response. Toxicol Appl Pharmacol 2010; 242: 318–325

[24] Yakubu Y, Adoum OA, Wudil AM, Ladan Z. Toxicity study of ethanol root extract of Terminalia macroptera Guill Perr (Combretaceae) and assessment of some heavy metals. Afr J Pure Appl Chem 2015; 9: 193–196

[25] Chassagne F, Haddad M, Amiel A, Phakeovilay C, Manithip C, Bourdy G, Deharo E, Marti G. A metabolomic approach to identify anti-hepato-carcinogenic compounds from plants used traditionally in the treatment of liver diseases. Fitoterapia 2018; 127: 226–236

[26] Loux JJ, DePalma PD, Yankell SL. Antipyretic testing of aspirin in rats. Toxicol Appl Pharmacol 1972; 22: 672–675

[27] Siegmund E, Cadmus R, Lu G. A method for evaluating both non-narcotic and narcotic analgesics. Exp Biol Med 1957; 95: 729–731

[28] Winter CA, Risley EA, Nuss GW. Anti-inflammatory and antipyretic activities of indo-methacin 1-(p-chlorobenzoyl)-5-methoxy-2-methyl-indole-3-acetic acid. J Pharmacol Exp Ther 1963; 141: 369–376

[29] Afzal M, Khan R, Kazmi I, Anwar F. Hepatoprotective potential of new steroid against carbon tetrachloride-induced hepatic injury. Mol Cell Biochem 2013; 378: 275–281

[30] Arbonnier M. Trees, shrubs and lianas of West African dry zones. ebook: Editions Quæ. 2019

[31] Diniz MA, Silva O, Paulo MA, Gomes ET. Medicinal uses of plants from Guinea-Bissau. In: van der Maesen LJG, van der Burgt XM, van Medenbach de Rooy JM editors The Biodiversity of African Plants. Dordrecht: Springer; 1996: 727–731

[32] Kerharo J, Adam J G. Traditional Pharmacopoeia of Senegal; Medicinal and Toxic Plants. Paris: Vigot Frères. 1974: 1011

[33] Nadembega P, Boussim JI, Nikiema JB, Poli F, Antognoni F. Medicinal plants in Baskoure Kourittenga Province Burkina Faso: An ethnobotanical study. J Ethnopharmacol 2011; 133: 378–395

[34] Pham AT, Dvergsnes C, Togola A, Wangensteen H, Diallo D, Paulsen BS, Malterud KE. Terminalia macroptera its current medicinal use and future perspectives. J Ethnopharmacol 2011; 137: 1486–1491

[35] Traore MS, Baldé MA, Diallo MST, Baldé ES, Diané S, Camara A, Diallo A, Balde A, Keïta A, Keita SM, Oularé K, Magassouba FB, Diakité I, Diallo A, Pieters L, Baldé AM. Ethnobotanical survey on medicinal plants used by Guinean traditional healers in the treatment of malaria. J Ethnopharmacol 2013; 150: 1145–1153

[36] Sanon S, Ollivier E, Azas N, Mahiou V, Gasquet M, Ouattara CT, Nebie I, Traore AS, Esposito F, Balansard G. Ethnobotanical survey and in vitro antiplasmodial activity of plants used in traditional medicine in Burkina Faso. J Ethnopharmacol 2003; 86: 143–147

[37] Traoré M. "Resort to traditional African pharmacopoeia in the new millennium", Case of herbal women of Bamako. CODESRIA 10th General Assembly 2002; 10–12

[38] Inngjerdingen K, Nergård CS, Diallo D, Mounkoro PP, Paulsen BS. An ethnopharmacological survey of plants used for wound healing in Dogonland Mali West. Africa. J Ethnopharmacol 2004; 92: 233–244

[39] Silva O, Viegas S, de Mello-Sampayo C, Costa MJP, Serrano R, Cabrita J, Gomes ET. Anti-Helicobacter pylori activity of Terminalia macroptera root. Fitoterapia 2012; 83: 872–876

[40] Silva O, Ferreira E, Pato MV, Caniça M, Gomes ET. In vitro anti-Neisseria gonorrhoeae activity of Terminalia macroptera leaves. FEMS Microbiol Lett 2002; 217: 271–274

[41] Silva O, Duarte A, Pimentel M, Viegas S, Barroso H, Machado J, Pires I, Cabrita J, Gomes E. Antimicrobial activity of Terminalia macroptera root. J Ethnopharmacol 1997; 57: 203–207

[42] Silva O, Duarte A, Cabrita J, Pimentel M, Diniz A, Gomes E. Antimicrobial activity of Guinea-Bissau traditional remedies. J Ethnopharmacol 1996; 50: 55–59

[43] Batawila K, Kokou K, Koumaglo K, Gbéassor M, de Foucault B, Bouchet P, Akpagana K. Antifungal activities of five Combretaceae used in Togolese traditional medicine. Fitoterapia 2005; 76: 264–268

[44] Traore MS, Diane S, Diallo MST, Balde ES, Balde MA, Camara A, Diallo A, Keita A, Cos P, Maes L. In vitro antiprotozoal and cytotoxic activity of ethnopharmacologically selected Guinean plants. Planta Med 2014; 80: 1–5

[45] Silva O, Barbosa S, Diniz A, Valdeira M, Gomes E. Plant extracts antiviral activity against Herpes simplex virus type 1 and African swine fever. Virus Int J Pharmacogn 1997; 35: 12–16