WORLD JOURNAL OF CURRENT MEDICAL AND PHARMACEUTICAL RESEARCH

www.wjcmpr.com ISSN: 2582-0222

Evaluation of In Vitro Antiplasmodial Activity of *Icacina oliviformis's* (Stems and Roots) and *Spondias Purpurea's* (Leaves, Barks And Roots) Extracts from Ziguinchor, Senegal

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ABSTRACT

This study was designed to assess phytochemical and antiplasmodial activities of *Icacina oliviformis's* and *Spondias purpurea's* extracts. *Icacina oliviformis* (poiret) Raynal (Icacinaceae) and *Spondias purpurea* L. (Anacardiaceae) are Senegalese traditional pharmacopoeia plants used to treat various diseases. These plants are used for treating malaria and related conditions in south Senegal.The following phytochemicals were detected into the two species: saponins, alkaloids, sterols, polyterpenes, polyphenols, flavonoids, tannins and free or combined quinones. As regards the evaluation of antiplasmodial activity against P. *falciparum* 3D7 strain, the ethanolic/water extract of *Icacina oliviformis* stems (SEWIO) displayed the best activity with an IC_{50} = 1.80 ± 0.51 µg/mL. Therefore, the same extract exhibits some degree of cytotoxicity with a CC_{50} = 78.30 ± 2.45 µg/mL. The antiplasmodial activity and the cytotoxicity of *Icacina oliviformis's* (stems and roots) and *Spondias purpurea's* (leaves, barks and roots) extracts are reported for the first time.

Key words:
Icacina oliviformis,
Spondias purpurea L., *in vitro,*
antiplasmodial, P. *falciparum,* cytotoxicity.

Article History:
Received On: 15.07.2019,
Revised On: 09.10.2019,
Accepted On:18.10.2019

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INTRODUCTION

Herbal medicines are used in many areas of pathology namely malaria, cancer, diabetes etc. Although global morbidity and mortality have significantly decreased, malaria, a parasitic infection of red blood cells, still kills an estimated 2,000 people a day, most of whom are children in Africa.

Two factors account for most of this decline: the increased distribution of insecticide-treated mosquito nets and the availability of highly effective treatments with artemisinin-based products[1]. However, malaria remains the most dangerous parasitic disease for humans. It is transmitted in 108 countries with about 3 billion people and, in 2010, there were an estimated 216 million patients and 655,000 deaths[2].

The situation has worsened because malaria parasites are becoming resistant to several antimalarial agents[3,4]. Medicinal plants have been used in almost all cultures as a source of medicine[5,6].

With the aim of discovering new natural active extracts against malaria parasites, *Icacina oliviformis* and *Spondias purpurea* were selected after an ethnopharmacological survey conducted on plants used for traditional treating of malaria in Senegal[7,8]. Miers[9]describes, in 1851, the family of Icacinaceae to more than 400 species grouped in about 54 genera. They are large rainforest trees, shrubs or lianas[10,11]. *Icacina oliviformis* is mostly found in west and central Africa[7,8].

The species *Icacina oliviformis* is used in Africa to treat various pathologies such as fever, malaria, asthenia, internal bleeding etc[12,13]. In Senegal the leaves of *Icacina oliviformis* are used in traditional medicine for treating diabetes[14].

The Anacardiaceae family contains about 77 genera and about 600 species, most of which fruits are drupaceous with resinous mesocarp. The species in this family are trees, shrubs or lianas with alternating, compound and imparipinnate leaves, mostly found in tropical to subtropical and temperate regions of the northern hemisphere. *Spondias* is a genus of this family and comprises about 17 described species, 7 of which are native to tropical America and 10 from South and Southeast Asia. Many species of this family are well known for their edible fruits and seeds (mangoes, pistachios, cashew nuts) [15,16].

In Nigeria, an infusion of shredded leaves of *spondias purpurea* is used to wash wounds, cuts and burns. The leaves of this tree therefore have antibacterial properties. In Jamaica, leaves are boiled to cure colds; they are also used to treat sore gums, diarrhea and dysentery. In Guatemala leaves decoctions are used for treating gastrointestinal disorders[17,18].

MATERIALS AND METHODS

PLANTS MATERIALS

Stems and roots of *Icacina oliviformis* and leaves, barks and roots of *Spondias purpurea* L. were collected in April 2016 from Diabyr and Kandé district respectively, region of Ziguinchor, Senegal. The plants were authenticated by Prof. E. Bassène, Pharmacognosy and botany Department, University Cheikh Anta Diop, Dakar, Senegal. Voucher specimens were deposited at the herbarium the Pharmacognosy and botany laboratory under respective numbers 2016/021 and 2016/022.

PLANTS EXTRACTION AND FRACTIONATION

The samples are cleaned and dried in the shelter of sun's rays at the ambient temperature of the laboratory (30°C). The dried drugs are crushed using a grinder (type Bradender OHG Duisburg). The fine powders (250 g) thus obtained after spraying were used as raw material to make the extractions. Successive depletion of the powder is achieved by solvents of increasing polarities (cyclohexane, acetone, dichloromethane, ethanol, ethanol/water and water). In fact, 250g of powder of each sample are introduced into a 5 L flask containing 3 L of solvent and then refluxed for 4 hours. Each solvent was evaporated to dryness to give a residue of mass *Icacina oliviformis* (stems and roots) and *Spondias purpurea* (leaves, barks and roots) (Table 1).

PHYTOCHEMICAL METHODS

Phytochemical screening of secondary constituents present in the plant extracts was carried out using methods adopted in similar surveys[19]. This quantitative and phytochemical analysis of these plants was determined as follows: Sterols and terpenoids (Lieberman's reagents); alkaloids (Bouchardat's/Valser-Mayer's/Dragendorff's reagents); flavonoids (concentrated HCl + magnesium ribbon); tannins (Stiasny's reagent, Formalin 30%, Concentrated HCl: 1/0.5); saponins (foaming test); polyphenols (reaction with ferric chloride ($FeCl_3$)); free or combined quinines substances (Borntraegen's reagent).

ANTIPLASMODIAL ASSAY

The antimalarial activity of extracts/compounds was evaluated against *P. falciparum* 3D7, using the fluorescence-based SYBR Green I assay approach in 96-well microplates as described by Smilkstein and al.[20]with some modifications. Positive control wells for each assay contained no inhibitor while negative controls contained Chloroquine (CQ). The CQ molecule was provided from World Wide Antimalarial Resistance Network (wwarn Network). Experiments were run in duplicate with both test and control drugs employed at varying concentrations. Stock solutions (extracts) were prepared in dimethyl-sulfoxide (DMSO) and diluted with culture medium to give a maximum DMSO concentration of 0.5% in a final well volume of 200 µL containing 1% parasitemia and 2.5% haematocrit. Extracts and negative control [Chloroquine (CQ)] were prepared by two-fold dilution, in a dosetitration range of 0.098-100 µg/mL, to obtain 11 concentrations each, in duplicate. The concentrations used for CQ were between 0.5 and 1000 nM. After 48 h incubation, the plates were subjected to 3 freeze thaw cycles to achieve complete hemolysis. The parasite lysis suspension was diluted 1:5 in SYBR Green I lysis buffer (10 mM NaCl, 1 mM Tris HCl pH8, 2.5 mM EDTA pH 8, 0.05% SDS, 0,01 mg/mL proteinase K and 10X SYBR Green I). Incorporation of SYBR Green I in parasite DNA amplification was measured using the Master epRealplex cycler® (Eppendorf, France) according the following program to increase the SYBR green incorporation: 90°C for 1 min, decrease in temperature from 90°C to 10°C for 5 min with reading the fluorescence 10°C for 1 min and a new reading at 10°C for 2 min. The IC_{50} was calculated by nonlinear regression using icestimator website 1.2 version: http://www.antimalarialicestimator.net/MethodIntro.htm.

CYTOTOXICITY ON HUVEC

HUVEC cells were cultured in Gibco™ RPMI 1640 medium (Life technologies, France) complemented with 10% Fetal Bovine Serum and 1 mM L-glutamine (Sigma-Aldrich, France) and incubated in 5% CO_2 at 37°C. The cytotoxicity of extracts was evaluated using the SYBR Green I assay as previously described. HUVEC were seeded in a 96-well plate at 100,000 cells/well and incubated for 24h to adhere. After discarding the old medium, the cells were incubated in the medium containing eight concentrations (0.78-100 µg/mL) of each extract in duplicate. After 48h incubation, cells were visualized using an inverted microscope to check their morphology or the cell viability. The medium was subsequently removed and replaced by lysis buffer without SYBR Green I and the plates were subjected to 3 freeze-thaw cycles. The cell lysis suspension was diluted 1:2 in SYBR Green I lysis buffer. The incorporation of SYBR Green I in cell DNA and the IC_{50} analysis were obtained as previously.

RESULTS

Table 1 gives us the yields of the different extracts. The yield is calculated as being the ratio of the mass of the extract on that of the starting dried powder.

Table 1: Extraction yields of the various extracts of *I. oliviformis* and *S. purpurea*

Extracts	Mass (g)	Yields (%)
Roots dichlorométhanic of *Icacina oliviformis* (RDIO)	0.88	0.95
Roots acetonic of *Icacina oliviformis* (RAIO)	0.74	0.80
Roots Ethanolic/water of *Icacina oliviformis* (REWIO)	2.15	2.33
Stems Ether of *Icacina oliviformis* (SEIO)	1.24	0.17
Stems dichlorométhanic of *Icacina oliviformis* (SDIO)	5.65	0.76
Stems Ethanolic/Water of *Icacina oliviformis* (SEWIO)	7.96	1.08
Stems Water of *Icacina oliviformis* (SWIO)	7.50	1.01
Leaves Ether of *Spondias purpurea* (LESP)	2.24	0.30
Leaves dichloromethanic of *Spondias purpurea* (LDSP)	6.82	0.94
Leaves Water of *Spondias purpurea* (LWSP)	76	10.50
Barks Cyclohexanic of *Spondias purpurea* (BCSP)	3	0.82
Barks Water of *Spondias purpurea* (BWSP)	62	17.12
Roots Water of *Spondias purpurea* (RWSP)	54	14.21

After the extraction of the secondary metabolites, a phytochemical screening (Table 2) was carried out in order to qualitatively determine the different types of families of molecules present in these extracts, then we tested these different extracts against *P. falciparum* 3D7 strain as well as their cytotoxicity against HUVEC. The test results are shown in Table 3.

Table 2: Results of the phytochemical screening of extracts of *I. oliviformis* and *S. purpurea*

Extraits	sterols and polyterpenes	polyphenols	flavonoids	tannins	free or combined quinones	alkaloids	saponins
RDIO	+	+	-	+	+	+	*
RAIO	+	-	-	-	-	+	*
REWIO	+	+	+	+	+	-	+
SEIO	+	+	-	+	+	+	*
SDIO	+	-	-	-	+	+	*
SEWIO	-	+	+	+	-	+	+
SWIO	-	-	+	-	-	-	+
LESP	-	+	-	+	-	-	*
LDSP	+	+	-	+	-	-	*
LWSP	+	+	+	+	+	+	-
BCSP	+	+	-	+	-	+	*
BWSP	+	+	+	+	+	-	-
RWSP	+	+	+	+	-	+	+

Table 3: Antiplasmodial activity and cytotoxicity of fractions of *I. oliviformis* and *S. purpurea*

	Extracts	*Plasmodium falciparum* 3D7 strain	HUVEC cells
		IC_{50} µg/mL **±** SD	CC_{50} µg/mL ± SD
1	RDIO	12.22**±**2.85	49.25**±**5.02
2	RAIO	17.50**±**4.25	>100
3	REWIO	>100	>100
4	SEIO	11.72**±**2.86	26.30**±**2.10
5	SDIO	25.59**±**4.07	47.50**±**3.12
6	SEWIO	1.80**±**0.51	78.30**±**2.45
7	SWIO	24.23**±**1.83	15.20**±**2.12
8	LESP	10.27**±**3.61	>100
9	LDSP	6.22**±**0.56	>100
10	LWSP	>100	>100
11	BCSP	>100	51.20**±**4.56
12	BWSP	54.89**±**6.04	>100
13	RWSP	>100	>100
14	CQ	5.92 ± 0.21	>100

CQ = chloroquine

DISCUSSION

In order to discover new natural active extracts against malaria parasites, *Icacina oliviformis* and *Spondias purpurea* were selected following an ethnopharmacological survey conducted on plants used in the traditional treatment of malaria in Senegal.

In this article we describe the extraction of *Icacina oliviformis* (stems and roots) and *Spondias purpurea* L. (leaves, barks and roots) and the examination of their antiplasmodial activities (Table 3). We retain in the table 1 that the stems of *Icacina oliviformis* and the barks and roots of *Spondias Purpurea* are very rich in polar compounds. According to WHO recommendations and previous works[21,22], the antiplasmodial activities of plants extracts were classified as follows: highly active extracts with an IC_{50}<5 µg/mL, promising activity between 5-15 µg/mL, moderate activity between 15-50 µg/mL and inactivity when IC_{50}> 50 µg/mL. When the cytotoxic concentration CC_{50}> 100 µg/mL, the extract is non-cytotoxic. On the basis of its WHO recommendations, we can say that the results of the seven extracts of *Icacina oliviformis* are in the range of inactivity (IC_{50}> 100 µg/mL) to highly active extracts (IC_{50} = 1.80 ± 0.51 µg/mL).

On the basis of this classification, we can say that SEWIO extract is a highly active extract with an IC_{50} = 1.80 ± 0.51 µg/mL. The SEIO and RDIO extracts are promising activity extracts with inhibitory constants equal to 11.72 ± 2.86 µg/mL and 12.22 ± 2.85 µg/mL respectively. However, the SDIO, SWIO and RAIO extracts are extracts with moderate activity. REWIO extract with an IC_{50} >50 µg/mL is an inactive extract.

We note that REWIO and RAIO extracts with cytotoxicity constants CC_{50} >100 µg/mL do not show on the one hand any cytotoxicity while showing respectively an inactivity and a moderate activity. On the other hand, the SEWIO extract which gave the best IC_{50} in the tests contains a certain level of cytotoxicity with a CC_{50} = 78.30 ± 2.45 µg/mL.

As for *S. purpurea*'s extracts, their activities vary from promising (IC_{50} = 6.22 ± 0.56 µg/mL) to inactivity (IC_{50}> 100 µg/mL). The both LESP and LDSP extracts have promising activity of IC_{50} = 10.27 ± 3.61 µg/mL and 6.22 ± 0.56 µg/mL, respectively. On the other hand, all the other extracts (LWSP, BCSP, BWSP and RWSP) showed inactivity (IC_{50}> 50 µg/mL, Table 3). All extracts are non-cytotoxic (CC_{50}> 100 µg/mL), with the exception of BCSP extract with a CC_{50} = 51.20 **±** 4,56 µg/mL. Among all the extracts, SEWIO is the only one which

has exhibited a better activity than chloroquine (CQ) (IC_{50} =5.92 ± 0.21 µg/mL) against P. *falciparum* 3D7 strain.

Phytochemically, a chemical characterization of *Icacina oliviformis and Spondias purpurea* showed in both species the presence of sterols, polyterpenes, polyphenols, flavonoids, tannins, free or combined quinones, alkaloids, and saponins (Table 2). Several studies have described the antiplasmodial effect of these secondary metabolites[23,24] which could explain the observed results on the activity of extracts of *Icacina oliviformis* and *Spondias purpurea.*

Numerous medicinal plants containing a wide variety of secondary metabolites have shown antiplasmodial activities[25-27]. The traditional use of *Icacina oliviformis* and *Spondias purpurea* for the treatment of malaria could be attributed to the presence of certain phytochemicals compounds that constitute the bioactive principles in the plants. In this study we will note that extracts show results varying promising inhibitions to inactivity. This pharmacologic potential is attributed to the phytochemical profiles of these extracts.

CONCLUSION

The different polar solvent extracts of *Icacina oliviformis* and *Spondias purpurea* exhibited wide spectrum antiplasmodial potencies against *Plasmodium falciparum* 3D7 strain. The SEWIO extract of the stems of *Icacina oliviformis* showed a strong inhibition on the P. *falciparum* 3D7 strain (IC_{50}=1,80 ± 0,51 µg/mL). The SEIO and RDIO extracts of *Icacina oliviformis* and the LESP and LDSP extracts of *Spondias purpurea* showed promising activity against P. *falciparum* 3D7 strain. These different results support the ethnomedical use of these plants in the treatment of malaria and associated symptoms.

ACKNOWLEDGMENTS

The authors thank the Senegalese government for the funding of this thesis project and Laboratoire de Chimiothérapie Antiparasitaire, UMR 8076 CNRS BioCIS, Université Paris-Sud, Université Paris-Saclay, 5 rue JB Clement 92296 Châtenay Malabry Cedex, France for bioactive tests.

CONFLICT OF INTEREST

The authors declare no conflicts of interest. The authors alone are responsible for the content of this manuscript.

REFERENCES

1. White NJ, Pukrittayakamee S, Hien TT, Faiz MA, Mokuolu OA, Dondorp AM. « Malaria ». The Lancet. 2014;383(9918):723‑735.
2. World Health Organization (WHO). Geneva2011, World Malaria Report 2011.
3. Giancarlo AB, Stephen A. Ward, Méchanisms of antimalarial drug resistance. Antimicrobial drug resistance. 2017;629-647.
4. Callaghan PS, Siriwardana A, Hassett MR, Roepe PD. *Plasmodium falciparum* chloroquine resistance transporter (PfCRT) isoforms PH1 and PH2 perturb vacuolar physiology. Malar J.2016;15: 186, https://doi.org/10.1186/s12936-016-1238-1
5. Ghulam M, Rawaba A, Asia A, Sumaira S, Amer J. Bioactive Compounds from Medicinal Plants and Their Importance in Drug Discovery in Pakistan. Matrix Sci Pharm. 2017;1(1):17- 26.
6. Mamatabala P, Gayatri N, Rajani KS. Review on Ethnomedicinal Plants of Odisha for the Treatment of Malaria. Int J Pharmacogn Phytochem Res. 2014-15;7(1):156-165.
7. Sarr OS, Perrotey S, Fall I, Ennahar S, Zhao M, Diop YM, *Icacina senegalensis* (Icacicaceae), traditionally used for the treatment of malaria, inhibits in vitro *Plasmodium falciparum* growth without host cell toxicity. Malar J. 2011;10:85.
8. Esien DO, Obiajunwa-Otteh JI. A combination of the leaves and tuber of *Icacina senegalensis* A. Juss (Icacinaceae) improves the antimalarial activity of the plant in mice. J coast life med. 2015;3(10):821 -825.
9. Miers. Icacinaceae. Annals And *Magazine* of *Natural History* (*Series 2*). 1851;8:174.
10. Kårehed J. Evolutionary studies in asterids emphasising Euasterids II. Acta Univ. Ups. Comprehensive summaries of Uppsala dissertations from the faculty of Science and technology 2002;761:50.
11. Villiers JF. Flore du Gabon. Icacinacées Olacacées Pentadiplandracées Opiliacées Octoknémacées. 1973;50.
12. Fay JM. *Icacina oliviformis* (Icacinaceae): A close look at an underexploited crop I. Overview and ethnobotany. Economic Botany 1987;41(4):512-522.
13. Sarr SO, Perrotey S, Fall I, Ennahar S, Zhao M, Diop YM,Candolfi E, Marchioni E. *Icacina senegalensis* (Icacinaceae), traditionally used for the treatment of malaria, inhibits in vitro *Plasmodium falciparum* growth without host cell toxicity. Malaria Journal. 2011;*10*(85): 10.
14. Manga A, Gassama A, Fall AD, Diatta K, Diatta W, Sy GY, Bassene E. Chemical and Pharmacological Study of antidiabetic fractions of *Icacina senegalensis* leaves (Icacinaceae). Med Afr Noir. 2013; 60(12):507-512.
15. Kpemissi-Amana E. *Les Anacardiaceae du Togo:* Etudes botaniques, écologiques et propriétés antifongiques, in Scieneces pharmacautiques. Université de Reims Champagne-Ardenne et Université de Lomé 2007;198.
16. Pinto AC, Braga WF, Rezende CM, Garrido FMS, VeigaJr. VF, Bergter L, Patitucci ML, Antunes OAC. Separation of Acid Diterpenes of *Copaifera cearensis* Huber ex Ducke by Flash Chromatography Using Potassium Hydroxide Impregnated Silica Gel.J. Braz. Chem. Soc.2000;11(4):355-360.
17. Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S. A tree reference and selection guide version. Agroforestree Database.2009;4:1-5. (http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp)
18. Philippine Medecinal Plant, Site web : http://www.stuartxchange.org/Sineguelas.html
19. Bidie AP, N'Guessan BB, Yapo AF, N'Guessan JD, Djaman AJ. Activités antioxydantes de dix plantes medicinales de la pharmacopée. Sci Nat. 2011;8:1 -11.
20. Komlaga G, Genta-Jouve G, Cojean S, Dickson RT, Mensah MLK, Loiseau PM, Champy P, Beniddir MA. Antiplasmodial Securinega alkaloids from *Phyllanthus fraternus*: Discovery of natural (+)-allonorsecurinine. Tetrahedron Letters. 2017;58:3754-3756.

21. 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.
22. Frausin G, Hidalgo AR, Braga R, Lima S, Kinupp VF, Ming LC, Pohlit AM, Milliken W. An ethnobotanical study of anti-malarial plants among indigenous people on the upper Negro River in the Brazilian Amazon. J Ethnopharmacol. 2015;174:238-252.
23. Cimanga KR, Lubiba NZ, Makila BMF, Tona LG, Kambu KO, Vlietinck AJ, Pieters L. Biological activities of arredoul jaune, a phytomedicine based ethanol extract from fresh roots of *pentadiplandra brazzeana baill.* (Pentadiplandadeae) used as an antidiarrhoeal drug in Kisangani-democratic republic of Congo. European j. biomed. pharm. Sci. 2018;5:130-139.
24. Atay I, Kirmizibekmez H, Kaiser M, Akaydin G, Yesilada E, Tasdemir D. Evaluation of *invitro* antiprotozoal activity of *Ajuga laxmannii* and its secondary metabolites. Pharm Biol. 2016;54:1808-1814.
25. Kau S, Gupta S, Sharma S, Dhar MK. The fungal endobiome of medicinal plants: a prospective source of bioactive metabolites. Medicinal plants and fungi: recent advances in research and development. Springer Link. 2017;167-228.
26. 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.
27. Essien DO, Obiajunwa-Otteh JI, Akuodor GC, Essien AD. Evaluation of the antimalarial potential of *Icacina senegalensis* Juss (Icacinaceae). Asian Pacific Journal of Tropical Medicine. 2014;7(Suppl 1):S469-S472.