Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2017, 9(2):51-55

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Phytochemical Study and in Vitro Activity Evaluation of Antisickling Effect of Two Plants Using in Senegalese Pharmacopeia

Sall Cheikh[1*], Ndoye Samba Fama[2], Dioum Mbayediaw[2], Seck Insa[2], Gueye Rokhayasylla[2], Gueye Papa Madièye[3] and Seck Matar[2]

[1]Laboratory of Chemistry, Research and Formation Unit of Health Sciences, Thies, BP 967 Thies University, Senegal
[2]Laboratoryof Organic and Therapeutic Chemistry, Faculty of Medicine, Pharmacy and Odontology (F.M.P.O.), Cheikh Anta Diop University (U.C.A.D), Dakar, Senegal
[3]Laboratory of Biochemistry, Faculty of Medicine, Pharmacy and Odontology (F.M.P.O.), Cheikh Anta Diop University (U.C.A.D), Dakar, Senegal

---

ABSTRACT

Sickle cell disease is a common haemoglobinopathy among black African populations. Approximately, 250,000 children are born each year in the world with this disease. The cases of death due to complications of sickle cell disease are mainly registered among children under five, teenagers and pregnant women. Currently, few conventional treatments are available on the market and they are not accessible for everybody. That's why, many sickle cell patients are turning to traditional medicine. Leptadenia hastata (Asclepiadacae) and Carica papaya (Caricaceae) are plants widely used by traditional healers in the management of sickle cell disea se.The objective of this study is to evaluate the antisickling activity of methanol extracts ofLeptadenia hastata roots and Carica papayagreen epicarp on sickle hemoglobin and identify theactive principles responsible of the observed activity.
Reversibility of sickle cell extracts was studied compared with controls (physiological mediumand arginine) in samples of blood from sickle cell homozygotes. Sickling was induced with a 2% solution of sodium metabisulfite. The evaluation was carried out every 30 minutes until 120 minutes.The extracts showed a dose-dependent activity on the reversibility of the sickling of red blood cells with 71% and 87% reversal in 120 minutes of incubation respectively, for L. hastata and C. papaya extracts. A phytochemical screening allowed to make a correlation between flavonoids and antisickling activity.The study justifies the traditional use of Leptadenia hastataroot and epicarp of Carica papaya in the treatment of sickle cell disease.

Keywords: Sickle cell disease; *Leptadenia Hastata*; *Carica papaya*; Flavonoids; Antisickling activity

---

INTRODUCTION

Sickle cell disease is a genetic disease caused by abnormal hemoglobin HbS which polymerizes under hypoxia conditions. This genetic disorder is the result of the substitution of glutamic acid by valine at the sixth codon of the globin gene. Thus, this hemoglobinopathy is widespread among black African populations with a prevalence of sickle cell trait from 10 to 40% depending on the country [1]. In Senegal, 10% are carriers of the sickle cell trait and in Democratic Republic of Congo it is 40%resulting in a prevalence of sickle cell disease ranging from 2% to 4% [2]. In addition, it is estimated that 250,000 children are born each year worldwide with sickle cell disease [3]. Sickle cell disease is the cause of a decrease in the solubility of the deoxy form of sickle hemoglobin (deoxy-HbS). The result is the polymerization of the deoxy HbS in the blood vessels leading to a vasoocclusion, responsible of most painful symptoms experienced during the disease. The polymerization of the deoxy-HbS is associated with the

activation of the Gardos channel (loss of K^+ and Cl^-) and significant dehydration of hemoglobin which causes an increase in the density of red blood cells [4], leading to decreased blood flow in organs and tissues [5]. The pathophysiology of sickle cell disease is complex, the most common manifestations are the adherence of red blood cells of the epithelium of blood vessels, acute pain episodes, pulmonary hypertension, chest pain, thromboembolism, inflammation,possibility of activation of the coagulation system [6]. The persistence of the polymerization of HbS is the cause of the deformity rigid cells and contribute to the long term, vaso-occlusive crises and destruction of the cells of the peripheral circulation [6]. Complications can be fatal for sickle cell subjects. The most exposed populations are children under five years, adolescents and pregnant women [1]. Sickle cell disease is, for all these reasons, a public health problem in Africa. Hydroxyurea, only available conventional treatment, is promoting the production of fetal hemoglobin and thus avoids the vaso-occlusive crises, the effects related to blood transfusion and acute crises of the chest. Prolonged use of hydroxyurea in sickle cell subjects, resulting in a significant reduction in mortality and morbidity [7]. However, much of the sickle cell turns towards traditional medicine since without access to hydroxyurea and other conventional treatments. Indeed, the practice of traditional medicine by use of medicinal plants is a long history in many cultures in Africa. According to Afolabi[8]two-thirds (2/3) of the world population particularly those living in developing countries use this traditional medicine for primary health care first line. In this traditional medicine, specific information on dosage and how effective treatments are still not data and differ from a traditional healer to another. Especially that the same portion of plant organ may be prescribed for several diseases.

Leptadenia hastata (Asclepiadacae) and *Carica papaya* (Caricaceae) are plants widely prescribed by traditional healers in the management of sickle cell disease. *Leptadenia hastata,*Asclepiadacae, is an herbaceous vine creeping stems. This is a widespread species in arid to semi arid regions particularly in those of south of Sahara. As against the *Carica papaya*, Caricaceae, is a plant of American origin and widespread today in tropical areas and inter tropical world [9]. The ripe fruit of Carica papaya, has already been proving its antisicklingeffect, but to our knowledge there has not yet been studies on the epicarp of this plant against sickle haemoglobin. Various organs of these plants have different therapeutic uses in different parts of the world ([8, 10-12]. The objective of this study is to compare the antisickling activity of *Leptadenia hastata* methanol root extract and the green epicarp of*Carica papaya*fruit on sicklecell and identify the active principles responsible of this activity.

MATERIALS AND METHODS

Chemicals and blood samples

Fresh blood was collected with full informedconsent from male and female sickle cell patients with confirmed sickle cell SS by Emmel test and electrophoresis. These patients have not been transfused or treated with hydroxyurea in the last six months. The age of patients ranged from 7 to 35 years. Sodium EDTA tubes were used for the collection and storage of the blood samples. The tests were done in the Laboratory of Biochemistry of the University Hospital of Fann. Chemical and biochemical products were obtained from different suppliers (Prolabo, Scharlau, Aldrich or Carlo Erba). The solvents were distilled before use.

Plants material

The roots of *Leptadenia hastata* used were purchased from traditional healers in Fassmarket in Dakar. The identification was carried out at the Laboratory of Pharmacognosy and Botany of the Faculty of Medicine, Pharmacy and Odontology at Cheikh Anta Diop University. Non ripe green fruit of Carica papaya are harvested at the nursery garden Forest Park Hann in Dakar. Peelings green fruit of *Carica papaya* and roots *Leptadeniahastata* were dried in a ventilated local sheltered from light, crushed and powdered.

Extraction of the plants material

The roots powder of*Leptadenia hastata* (100 g) is introduced into an Erlenmeyer flask and macerated in 200 ml of methanol for 48 h. The macerated is filtered and the solid residue is taken up in 200 ml of methanol. After three extractions, the various extracts are combined, dried and the solvent evaporated using a rotary evaporator. The crude extract obtained is collected and weighed. The same protocol was used to make methanol extraction of the powder green fruit epicarp of *Carica papaya*. The extracts obtained are stored in a refrigerator at 4°C until use.

Phytochemical screening

Phytochemical screening is a qualitative test to highlight the presence or absence of chemical groups in a vegetable product. The researched chemical groups in the methanolic fractions of the two plants are: alkaloids, saponins, tannins, anthracene and flavonoids by using the standard procedure described in [10].

Antisickling activity

The "in vitro" test used to evaluate the activity of methanol extracts of the root powder of *Leptadenia hastata* and the epicarp of *Carica papaya* is the Emmel test, according to a protocol adapted in [13]. The blood of sickle cell SS confirmed patients, is centrifuged for 5 minutes at 3,000 rpm three times to remove the supernatant serum. There after 20 µl of the remaining red blood cells are mixed with 20 µl of 2% metabisulphite sodium solution to induce sickling. To this mixture, 20 µl of the methanol fraction solutions were added with concentrations of0.05 and 0.5 mg/ml. Physiological medium and arginine were used respectively as negative and positive controls. The assembly is placed between slide and cover slip. Four independent studies are performed and each experiment is carried out in duplicate. Morphological analysis is performed using an optical microscope immersion. Every 30 minutes up to 120 minutes, reading is performed to determine the percentage of residual sickle hemoglobin. The results obtained are compared with those obtained with arginine used as positive reference and the physiological mediumused as a negative reference.

Data analyses

The results of antisickling activities were evaluated in percentage of residual sickle. For the negative control, since the number of sickle cells increases with time, it was considered that the rate of 100% correspond to the number of sickle cells obtained in 120 minutes. For samples tested, this number decreases over time so the rate of 100% is the number of sickle cells at the initial time T0. The evolution of the percentage of the residual sickled in function of time is given by the following equation:

$$PDR = \frac{\text{Mean of sickles at Tx}}{\text{Mean of sickles at T0}} \times 100$$

PDR = Percentage of residual sickles
Tx = 0, 30, 60, 90 et 120 minutes
T0 = initial time

RESULTS AND DISCUSSION

Results

The yields of methanol extractions are almost similar, 5% and 5.25% for *C papaya*and *L. hastata* respectively. Phytochemical screening (Table 1) carried out by measuring the foam index for saponins and by thin layer chromatography for other chemical groups shows that all the searched groups are present in varying degrees in the methanolic fraction of *C. papaya*. Then for the methanolic fraction of *L. hastata*, only tannins and flavonoids are present.

Table 1: Phytochemical screening

	Tannins	Flavonoids	Antracenics	Alkaloids	Saponins
C. papaya	+	+	+	+	+
L. hastata	+	+	-	-	-

Figures 1 and 2 show the results of antisickling activities of methanolic extract fractions of *L. hastata* and *C. papaya*at respectively the concentration of 0.05 and 0.5 mg/ml. These results show that both methanol fractions studied, have an effect on sickled red cells in comparison with the negative control for which the number of sickle cells increases over time and arginine considered as positive control. The methanol extracts have greater antisickling effects than arginine in both concentrations. The methanol extract of *L. hastata* seems to be available in the first minutes than *Carica papaya*. Indeed, for a concentration of 0.05 mg/ml reversibility of sickling of *L. hastata* is 46%, 48%, 61%, while that of *C. Papaya* is 36%, 47% and 48% for respectively 30, 60, 90 minutes incubation. This observation is valid in the first 60 minutes in a concentration of 0.5 mg/ml. For longer incubation time, the methanolic extract from *C. papaya* takes over and provides reversibility of percentages of the most significant sickling in both studied concentrations. Reversibility is 83 and 87% at 120 minutes incubation for respectively 0.05 and 0.5 mg/ml compared to 70 and 71% for *L. hastata*. A dose dependent effect was also observed. Indeed, as the concentration increases more reversibility of sickling increases.

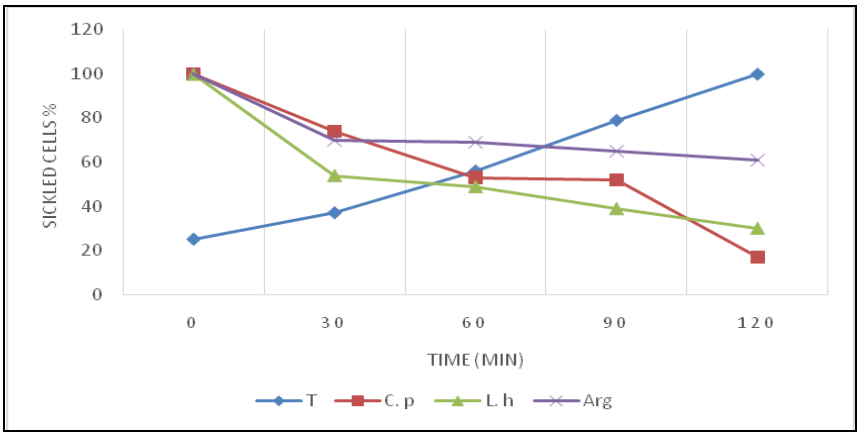

Figure 1: Antisickling activities of methanolic extracts of *L. hastata* and*C. papaya* at 0.05 mg/ml; T = negative control; C. p = *C. papaya*; L. h = *L. hastata*; Agr = arginine

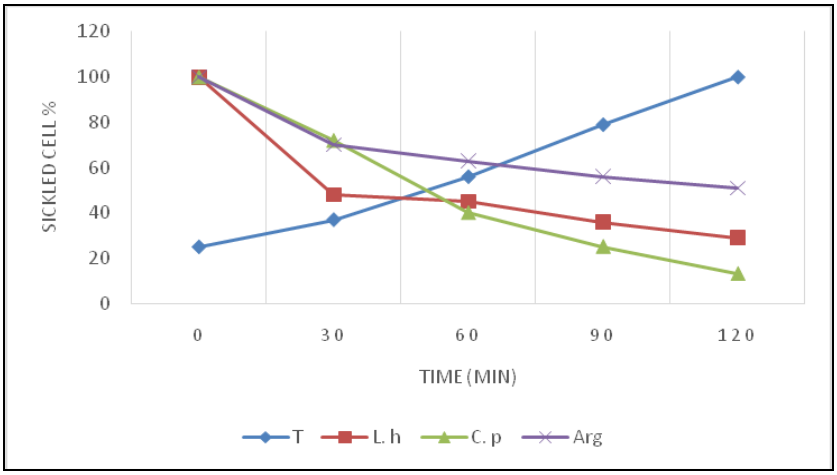

Figure 2: Antisickling activities of methanolic extracts of *L. hastata* and *C. papaya* at 0.5 mg/ml. T = negative control; C. p = *C. papaya*; L. h = *L. hastata*; Agr = arginine

Considered as the first genetic disease worldwide, sickle cell disease is still struggling to find a long-term conventional treatment with the exception of one drug recognized drug hydroxyurea in the management of sickle cell disease by "the US FDA." In Africa, many works have been developed in Herbal Medicine in order to develop traditional medicine which is less expensive and more accessible to the populations. Thus the FACA [14], the CIKLAVIT [15] and the MX1520 [16, 17] were prepared and some of them are now subject of clinical trials. It is in an effort to better develop this research on the management of sickle cell anemia. In this perspective, methanol extracts of *L. hastata* and *C. papaya* were tested on the SS type sickle. The observed activity of these fractions is due to the presence of the polar secondary metabolics. The most important activities of the fraction of *L. hastata* in the first minutes could be explained by the presence in smaller amounts of secondary metabolites. This would make these extracts more soluble and quickly metabolized. The synergistic effect of different secondary metabolites present in the extract from *C. papaya* is the cause of the reversibility of 87% of the sickling of red blood cells within 2 hours of incubation. This result is close to that obtained in Majisola study [9] of the activity of fermented fruit papaya on sickle. The activity of the extracts of these two plants is mainly due to the presence of flavonoids which are true antioxidants. They fight against reactive oxygen species produced during painful crises of sickle cell anemia [18]. In the case of *C. papaya*, it was reported that vitamin C, beta-carotene, citric acid, lycopene, methionine, alanine etc. present in the fruit would be responsible for the antioxidant activity papaya [19]. Furthermore Mojisola et al. [9] reported that amino acids such as tyrosine, phenylalanine, glycine etc., are responsible for the antisickling activity of*C. papaya fruit*. Arginine which has a less reversibility of the sickling of sickle cells as methanolic extracts. The results are respectively 39% and 49% of sickling reversibilityat 0.05 mg/ml and 0.5 mg/ml in 120 minutes of incubation. In addition, arginine is recommended in the treatment of sickle cell disease. Indeed, it is the

precursor of nitric oxide, a vasodilator of blood vessels which reduces the crises of vaso-occlusion by acting on endothelial functions [20].

CONCLUSION

The results obtained in this study have shown that the traditional use of *Leptadenia hastata*roots and the green epicarpof *Carica papaya* fruit in the treatment of sickle cell disease is well justified. For its active secondary metabolites against sickle hemoglobin, the papaya fruit could be recommended as a dietary supplement for sickle cell. In Senegal, some communities use the leaves of *Leptadenia hastata* in their cooking. A study of the effect of the combination of these two extracts on sickle cell SS as well as the study of their cytotoxicity and structural determination of the active metabolites of *Leptadenia hastata* and epicarp fruit of *Carica papaya* responsible of the antisickling activity is ongoing.

ACKNOWLEDGEMENTS

The authors of this article have their thanks and sincere gratitude to the Senegalese Ministry of Higher Education and Research for funding this project through the FIRST and The University of Thies for the financial support via the FAAR.

REFERENCES

[1] WHO. Regional comity of Africa. Sickle cell disease: a strategy for African regions of WHO.**2010**.
[2] I Diagne; ND Diagne-Gueye; H Signaté-Sy;B Camara; P Lopez-Sall. *MédecineTropicale*. **2003**, 63(4-5), 513-520.
[3] MT Tim. *Am Soc Hematol*. **2008**, 1, 193-196.
[4] FLucia De. *Medit J Hemat Infect Dis*, **2009**, 1 (1).
[5] MH Steinberg. *N Engl. J. Med*., **1999**, 340, 1021-1030.
[6] L Giuseppe; S Simona; C Patrizia; T Luciana; S Valerio De. *Haematologica*, **2001**, 86, 1236-1244.
[7] M HSteinberg; F Barton; O Castro; C H Pegelo. *JAMA*, **2003**, 289,1645-1651.
[8] SI Afolabi; OI Osikoya; F ODorcas; P IUsoro; OD Ogunleye; T Bisi-Adeniyi; OA Adeyemi; TB Adekeye. *J Altern Complem Med* **2012**, 12, 262-271.
[9] CO Mojisola; AE Adebolu; MD Alani. *J Nat Prod*, **2008**,1, 56-66.
[10] M Seck; C Sall; PM Guèye; I Seck; MD Dioum; Z Lembachar; RS Guèye; D Fall; M Fall; TND Dièye. *Int J Biol Chem Sci*,. **2015**, 9(3), 1375-1383.
[11] T Oduola; OT Idowu; SI Bello; AF Adeniyi; OE Ogunyemi. *Asian J Pharm Clin Res*, **2012**,5, (3), 77-81.
[12] C Baskaran; V Rathabai; S Velu; K Kumaran. *Asian Pacific J Trop Dis*, **2012**, S658-S662
[13] AN Imaga; GO Gbenle; VI Okochi; SO Akanbi; SO Edeoghon; V Oigbochie; MO Kehinde; SB Bamiro. *Afr. J. Biochem. Res*., **2009**, 3(4), 102-106.
[14] B Ouattara; O Jansen; L Angenot ; IP Guissou ; M Frederich;P Fondu; M Tits. *Phytomedicine*., **2009**, 16 (2-3), 125-129.
[15] A NImaga; EC Chukwu; A Blankson; O Gbenle. *J herbal med*., **2013**, 3, 137-148.
[16] C Zhang; X Li; L Lian; Q Chen; O Abdulmalik; V Vassilev; S C Lai; T Asakura. *British J Haematol*, **2004**,125, 788-795.
[17] A NImaga; AA Olusegun. *J Pharmacog Phytother*., **2010**, 2(7), 97-102.
[18] H Railson; FO Michel; EFF Aline; H Priscila; JDN Aguinaldo; SSL Maria. *Revista Bras Hematol Hemoterapia*, **2013**, 35 (1), 52-55.
[19] JA Duke. Handbook of phytochemical constituents of GRAS herbs and other economic plants (and Database) Boca Raton, FL. CRC Press, Inc., **1992**.
[20] CR Morris. *Hematol Oncol Clin N Am*., **2014**, 28(2), 301-321.