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Research Article
Eclipta alba (L.) Hassk. (Asteraceae) Methanolic Extract Phenolics contain Behavioural Effects on NMRI Mice

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Abstract

Background and Objective: Many plants of the Asteraceae family are used in the symptomatic treatment of several neuropsychiatric disorders. Among these, *Eclipta alba* (L.) Hassk., is also known as *Eclipta prostrata* is well known in traditional medicine. This study aimed to evaluate the psychotropic and biological properties of *Eclipta alba* (L.) Hassk., extracts. **Materials and Methods:** A methanolic extraction was performed after harvesting the whole plant. The extract was used for spectrophotometric determination of total polyphenols and total flavonoids, evaluation of antioxidant activities using ABTS, DPPH and FRAP methods, determination of acute toxicity (5000 mg kg^{-1} b.wt.) and also psychotropic effect on mice (50 mg kg^{-1}). **Results:** Regarding the polyphenolic compounds contain, the methanolic extract of *Eclipta alba* (L.) Hassk., gave 18.85±0.61 mg EAG/100 mg extract in total phenolic and 6.38±1.05 mg EQ/100 mg extract in total flavonoid. The toxicity test of the methanolic extract of *Eclipta alba* in mice established an LD_{50} greater than 5000 mg kg^{-1} b.wt. Concerning the behavioural study, the methanolic extract of *Eclipta alba* shows antidepressant activity. **Conclusion:** This study showed that the extract of *E. alba* has a pharmacological effect and justifies its use in the traditional medicine of Burkina Faso.

Key words: *Eclipta alba* (L.) Hassk., Burkina Faso traditional medicine, pharmacological capacity, behaviour, antioxidant activities, phenolic compounds, neuropsychiatric disorders

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Neuropsychiatric disorders are diseases group that affect human life and social life[1]. These conditions disable the individual and affect those around them. Mental and neurological disorders account for 13% of the total burden of disease in the world[1]. These disorders affect all categories of people, races, sexes and ages[2]. Epilepsy is one of the most common neurological disorders affecting over 50 million people worldwide[1]. A high prevalence has been observed in Africa where about 75% of patients do not receive adequate treatment[3]. In Burkina Faso, 17.5% of disability cases are caused by neuropsychiatric disorders[1].

Several molecules are used in the treatment of these neuropsychiatric disorders[4,5]. However, these modern treatments are expensive, complex and inaccessible for African populations in rural areas[1,4]. Many of these psychoactive molecules are of plant origin[1], which could justify the use of plants in traditional African medicine to treat neuropsychiatric diseases[6,7].

In Burkina Faso, medicinal plants are widely used by the population. Eclipta is a genus of plant that belongs to the tribe *Heliantheae,* itself belonging to the subfamily Asteroideae[8,9]. *Eclipta alba* is a herbaceous plant well known in traditional Asian and African medicine[10]. In traditional Asian medicine, its extracts are used against snake bites, to grow hair, gastrointestinal disorders, hepatoprotection, neuroprotection etc.[8,10,11]. In traditional medicine, this plant is used for its neuropharmacological profile. In Burkina Faso, *E. alba*, a traditional plant with the local name *Muso fii* is used to treat microbial diseases, infantile mycosis, epilepsy and infantile convulsions[9,12]. In Burkina Faso, the flora is in the edition. Scientific data on *Eclipta alba* use in neuropsychiatric disorders treatment remains very insufficient.

In the way contributing to this flora of Burkina Faso edition, the present study aimed to evaluate the neuropharmacological capacity of *Eclipta alba* (L.) Hassk., extracts using mice.

MATERIALS AND METHODS

framework of the study: An ethnobotanical survey was conducted in the city of Bobo-Dioulasso, Burkina Faso. Biological activities were carried out at the Laboratory of Research and Teaching in Animal Health and Biotechnology (LARESBA) of the Training and Research Unit in Sciences and Techniques of the Nazi BONI University (UNB) and the Health Sciences Research Institute (HSRI). This study was conducted at the Department of Applied Biology and Modeling of Biological Systems (BAMSB), Pharmacology Laboratory, Burkina Faso, in February to June, 2021.

Biological materials

Plant material: The whole plant of *Eclipta alba* (L.) Hassk., was collected during October, 2020 in the classified forest of Denderesso in Bobo-Dioulasso at the following geographical coordinates: 11°13'32.6"N and 004°25'57.2"W. It was authenticated by a botanist from Nazi BONI University before harvesting. Then, the whole plant was washed and dried in the laboratory in the shade at room temperature and pulverized with an aluminium mortar. The resulting powder was packaged and labelled in zipper-lock bags which were finally used for the various activities.

Animal material: A total of 54 NMRI (Naval Medical and Research Institute) mice, were obtained from the animal house of the Centre International de Recherche Developpement sur l'elevage en zone Subhumide (CIRDES) in Bobo Dioulasso. Adult male and female mice approximately 8-12 weeks old and weighing between 29 and 39 g were used for the different tests. Mice were kept under a temperature of 22±3°C with alternating light and dark every 12 hrs and free access to food and water. Mice were treated following the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare Act from Burkina Faso and all procedures complied with the European Council Directive of 24 November, 1986 (86/609/EEC). All evaluations were performed between 9 and 16 hrs.

Chemical reference compounds: Tramadol and scopolamine as reference substances were purchased from a local pharmacy.

Solvents and reagents: All solvents were of analytical grade. An Agilent Cary 60 UV-Vis spectrophotometer was used for all spectrophotometric measurements. Ascorbic acid, ferric chloride ($FeCl_3$), Aluminium Chloride ($AlCl_3$), potassium acetate, quercetin, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2, 2'-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid (ABTS), Folin-Ciocalteu reagent, gallic acid, sodium carbonate and methanol were obtained from Sigma Chemical Co. (St., Louis, MO, USA). Millipore deionized water was used throughout. Thiazolyl blue tetrazolium bromide (Sigma Aldrich, USA), Dimethyl sulfoxide (Sigma Aldrich, USA).

Extraction: Extraction was done with soxhlet and repeated 7 times to obtain 100 g of plant material powder. About 15 g of sample powder was loaded into extraction cartridges with 200 mL of methanol for at least 4 hrs at a constant temperature of 65°C. After recovery of the solvent, the extract was concentrated, collected in a Petri dish and dried under ambient laboratory conditions.

Determination of polyphenolic compounds

Quantification of total phenolic compounds: The estimation of total extractable phenolic compounds was performed by the Folin-Ciocalteu method described by Meda *et al.*[13]. The sample solution was diluted to one-hundredth of the stock solution. Three tubes were used into which a 0.125 mL volume of the diluted extract solution plus a 625 µL volume of 0.2 N Folin-Ciocalteu reagent was introduced and incubated for 5 min. A 0.5 mL volume of 75 g L^{-1} sodium carbonate solution in distilled water was then added and the mixture was incubated for 2 hrs.

Another tube with 125 µL of distilled water, 125 µL of Folin-Ciocalteu reagent and 0.5 mL of sodium carbonate was used for the blank. The reading was taken at 760 nm using a spectrophotometer UV-Visible after incubation.

The standard calibration curve was plotted using gallic acid (0-200 mg L^{-1}) ($y = 0.004668x+0.034$, $R^2 = 0.9991$). A total of three readings were taken for every extract and the result given was an average of these analyses. The results were expressed as mg gallic acid equivalent per 100 mg dry extract (mg GAE/100 mg extract).

Quantification of total flavonoids: The method used for the estimation of the flavonoid content in the plant extract was as described by Meda *et al.*[13]. The sample solution diluted to the hundredth was used to operate. Four tubes were prepared in which 0.625 mL of the diluted sample solution was introduced and then we added to the first three tubes a volume of 0.625 mL of 2% $AlCl_3$. The fourth tube considered as the control received 0.625 mL of methanol. The four tubes were incubated for 10 min in the dark. Quercetin (0-100 mg L^{-1}) was used as a standard for the development of the calibration curve ($y = 0.01259x$, $R^2 = 0.9990$). After incubation three readings are taken using a spectrophotometer at 415 nm wavelength, the result given was an average of the three. The results were expressed as mg quercetin equivalent (QE) per 100 mg of extract (mg QE/100 mg).

Biological activities

Antioxidant activity

Anti-radical activity by the DPPH• radical inhibition method: In three test tubes, 375 µL of the 1:100 diluted solution and 750 µL of DPPH solution (20 mg L^{-1}) were introduced and then incubated for 15 min in the dark. A blank was prepared with 375 µL of the sample and 750 µL of methanol. Absorbances and concentrations were read using a spectrophotometer at 517 nm against a standard ($y = -2.224.10^{-2}x+0.348$, $R^2 = 0.9966$) obtained from ascorbic acid. The method used is the one described according to the protocol of Meda *et al.*[13].

Reducing power by ABTS•+ method: A methanolic solution (10 mg mL^{-1}) is diluted to the hundredth in distilled water. Ten microliters of the sample (diluted solution) was taken and then mixed with 990 µL of freshly prepared ABTS•+ solution. The mixture was incubated in the dark for 15 min. Absorbances and concentrations were read 3 times at a wavelength of 734 nm in a spectrophotometer against a standard curve established from ascorbic acid[13]:

$$y = -7.874.10^{-4}x+0.709,\ R^2 = 0.9993$$

Reducing power by FRAP method: The dilution diluted to the hundredth from the stock solution was used to operate. In 3 test tubes 0.5 mL of the diluted solution was introduced and 0.5 mL of distilled water in another tube for the blank. To these different tubes, a volume of 1.25 mL of phosphate buffer (0.2 M, pH 6.6) and then a volume of 1.25 mL of potassium hexacyanoferrate [$K_3Fe(CN)_6$] were added. The whole is heated in a water bath at 50°C for 30 min. After this operation, 1.25 mL of trichloroacetic acid (10%) was added and the mixture is centrifuged at 3000 rpm for 10 min. About 625 µL of supernatant was removed from each tube and added to tubes containing 625 µL of distilled water. About 125 µL of freshly prepared Trichloroferrate [$FeCl_3$ (0.1%)] was added to the resulting mixture. The resulting solution was stirred and then run on a spectrophotometer for a series of 3 absorbance and concentration readings at a wavelength of 700 nm against a standard ($y = 3.270.10^{-3}x$, $R^2 = 0.9990$) established from ascorbic acid. The method used was described according to the protocol in Meda *et al.*[13].

The results of the antioxidant activities are determined by the following formula[13]:

$$C = \frac{c \times D}{M \times C_i}$$

- C = Concentration of free radical scavenging compounds in µmol AAE/g extract or fraction
- c = concentration of the sample read on the standard curve
- D = Dilution factor of the sample (100) to be determined
- C_i = Initial concentration of the sample solution to be determined (10 mg mL^{-1})
- M = Molar mass of ascorbic acid (176.1 g moL^{-1})

Acute toxicity study: The toxicity study was conducted according to Iniaghe *et al.*[14]. In total there were 4 groups of mice divided into 2 groups according to weight and sex and each group contained 3 animals. The mice were kept fasted for 18 hrs before the start of the experiment. The control groups (according to sex) were given water and the others were given the methanolic extract of *Eclipta alba* at 5000 mg kg^{-1} b.wt. The administration of the extract was done by gavage using a tube. After administration, the animals were monitored for behavioural changes and mortality. Animals were followed for two weeks with weight gain on days 0, 7 and 14.

In the end, all animals were sacrificed and organs such as liver, spleen, kidneys, lungs and heart were observed for morphological changes and weighed.

Behavioural test

Administration of the extract: The extract was prepared daily and administered by gavage for 14 consecutive days. For each test, mice have divided into three groups (n = 6) and received treatment according to the following distribution: The control group received physiological water, the positive control group received tramadol (20 mg kg^{-1}) and the last group received 50 mg kg^{-1} of *Eclipta alba* extract. On day 14, mice in both groups except the control group received 0.7 mg kg^{-1} of scopolamine hydrobromide by gavage 1 hr after the last treatment. Behavioural tests were performed 30 min after scopolamine administration. The anxiolytic, antidepressant and anti-amnestic properties of the extract were evaluated.

Y-maze test: This test assesses the short-term memory of the mouse. The maze has three arms (Fig. 1a). The mouse at the end of one arm can move freely in the maze for 8 min. An entry was considered when the animal's hind legs entered the arm completely. Spontaneous alternation behaviour was defined as consecutive entry into all three arms. It reflects spatial working memory, which is a form of short-term memory[15,16].

Elevated cross maze test: The elevated cross maze is a commonly used test for the assessment of anxiety, exploration and memory behaviours in laboratory animals[15,17-19].

This test was used to assess the effect of extracts on anxiety and memory in mice. The maze used consisted of four arms, including two open arms (49×10 cm) and two closed arms (49×10×30 cm), elevated to a height of 50 cm from the ground (Fig. 1b). For the assessment of the anxiety state, mice were individually placed in the centre of the maze and the time spent on the open arms and the number of entries into these arms were recorded for 5 min. After each mouse had passed through, the maze was thoroughly cleaned. The time spent and the number of entries into the open arms are indices of anxiety[21].

Forced swimming test: The antidepressant activity of the extract was evaluated using the forced swimming method previously described[15,20]. Briefly, mice forced to swim in a confined space from which they cannot escape will, after an initial period of vigorous activity (swimming), tend to adopt a characteristic posture of immobility in which they remain passively floating in the water making only movements necessary to keep their heads above water. This immobility reflects a state of despair and mental depression. Antidepressant treatment will tend to reduce this immobility time. The test set-up consists of a 35 cm high, 30 cm diameter cylindrical vessel containing 25 cm of water at 26±1°C

Fig. 1(a-c): Materials used for the evaluation of mice behaviour, (a) Y-Maze, (b) Elevated plus maze and (c) Forced swimming test apparatus

(Fig. 1c). In the pretest session, each mouse was immersed in a 15 min swim. The test was performed 24 hrs after the pretest session. The assessment was performed 30 min after scopolamine administration. Each animal was individually subjected to swimming for 6 min.

The following behavioural responses were recorded: Immobility time (time floating with minimal movement to keep the head above water) and swimming time (time spent with active swimming movements).

Statistical analysis: Results were expressed as Mean±Standard Deviation (SD). Data entry and analysis were done with Microsoft Word 2010 and Excel 2016. Graph Pad Prism, 2016 was used to analyze the behavioural test data. Results were considered significant at $p<0.05$.

RESULTS

Quantification of polyphenolic compounds: In this study, the content of total polyphenols and flavonoids in the methanolic extract was evaluated. The Folin-Ciocalteu method was used for the quantification of total polyphenols and the aluminium chloride method for flavonoids. Quantification of polyphenolic compounds in the methanolic extract of *Eclipta alba* resulted in a total polyphenol content equal to 18.85±0.61 mg EAG/100 mg. The content of total flavonoids was evaluated at 6.38±1.05 mg TEQ/100 mg.

Biological activities

Antioxidant activity: In the body, various types of oxidation take place and lead to the oxidation of free radicals. The diversity and specificity of antioxidants (radical scavengers, electron or hydrogen atom donors) make it necessary to evaluate the antioxidant activity by various methods. We note that the methanolic extract of *Eclipta alba* has antioxidant activity (Table 1). However, it is weak compared to the references (Trolox and Quercetin).

Effect of acute toxicity: The acute toxicity evaluation showed no mortality after oral administration of *Eclipta alba* extract at a single dose of 5000 mg kg^{-1} b.wt. Similarly, no changes in the general appearance of internal organs such as the heart, lungs, liver, spleen and kidneys were observed after the autopsy of the animals. It should also be noted that the relative weight of the organs was not changed in the treated groups compared to the control group. Compared to the weight on day 1, an increase in body weight was observed for all animals (treated and control groups) after the two weeks (Table 2).

Effect of the extract on mice

Estimation of the anti-amnesia effect: Mice have an innate tendency to explore new environments. The Y-maze was used to assess memory. At the end of the test, a slight increase in the spontaneous alternation of the tramadol group

Table 1: Results of the antioxidant capacity of *Eclipta alba*

		Methods		
Species	Part used	DPPH (µmoL EAA/g)	ABTS (µmoL EAA/g)	FRAP (µmoL EAA/g)
Eclipta alba	Whole plant	289.38±19.85	5529.05±181.49	989.85±105.62
Quercetin	Reference	646.85	14671.59±534.73	2211.24±36.17
Trolox	Reference	785.99	8137.61±229.49	5991.29±75.56

Data were expressed as Mean±SD

Table 2: Weekly body weight and relative organ weight after 2 weeks

		Males		Females	
Parameters		Control	Test	Control	Test
Body weight (g)	1st day	32.73±0.09	30.60±0.82	36.60±0.20	38.10±0.20
	7th day	40.11±1.11	36.92±0.40	36.22±0.83	37.79±1.76
	14th day	44.48±3.37	40.30±1.62	37.80±1.59	41.19±4.45
Weight gain	14th day	11.75	9.70	1.20	3.09
Mortality		0.00	0.00	0.00	0.00
Relative weight of organs (%)	Heart	0.38±0.02	0.35±0.03	0.38±0.08	0.40±0.06
	Lungs	0.72±0.18	0.58±0.05	0.51±0.09	0.56±0.02
	Liver	3.40±0.42	3.13±0.44	2.74±0.29	3.10±0.35
	Rate	0.38±0.06	0.31±0.03	0.47±0.04	0.44±0.03
	Kidneys	0.89±0.07	0.89±0.06	0.72±0.11	0.57±0.20

Data were expressed as Mean±SD

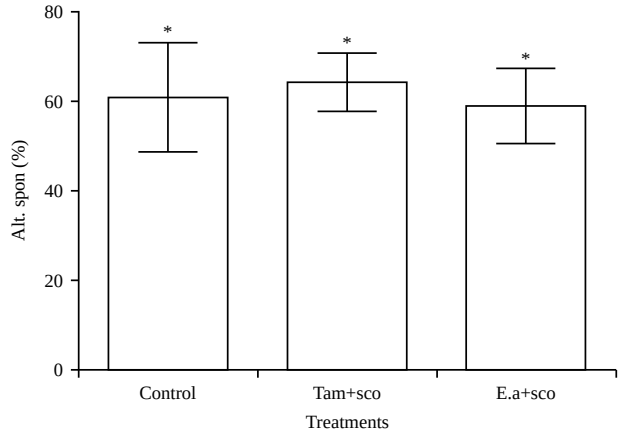

Fig. 2: Anti-amnestic effect of methanolic extract of *E. alba* (50 mg kg^{-1} b.wt.)
E.a+sco: *Eclipta alba*+Scopolamine, Tram+sco: Tramadol+Scopolamine and *p<0.05, significant difference compared to control group

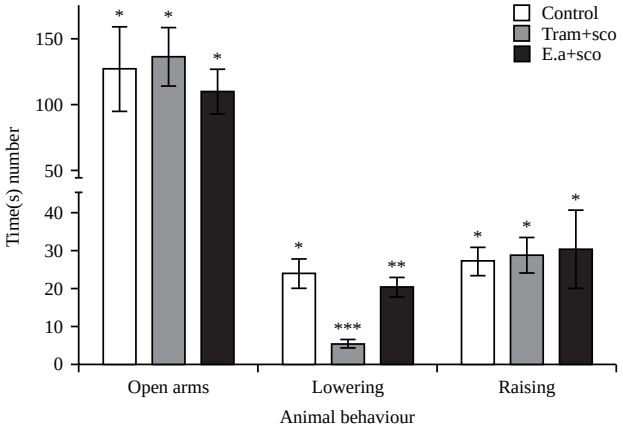

Fig. 3: Anxiolytic effect of *E. alba* extract (50 mg kg^{-1} b.wt.)
E.a+sco: *Eclipta alba*+Scopolamine, Tram+sco: Tramadol+Scopolamine, *p<0.05, **p<0.01 and ***p<0.001, significant difference compared to control group

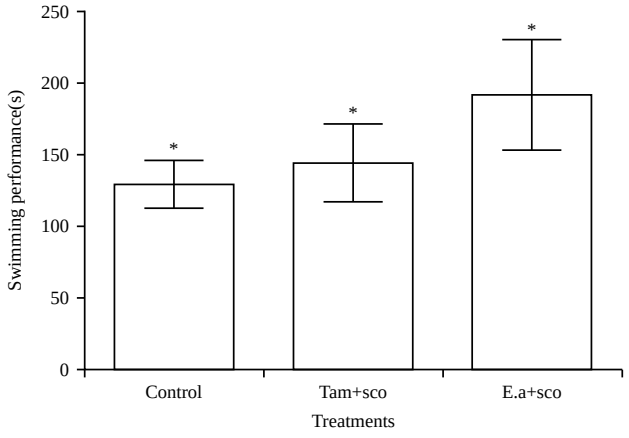

Fig. 4: Antidepressant effect of *E. alba* (50 mg kg^{-1} b.wt.)
E.a+sco: *Eclipta alba*+Scopolamine, Tram+sco: Tramadol+Scopolamine and *p<0.05, significant difference compared to control group

(Tram+sco) compared to the control group was observed (Fig. 2). This result indicated that the animals have an ability to the memory of the arm immediately explored before the current (arm from which it exits). The group receiving the methanolic extract of *Eclipta alba* (E.a+sco) showed a non-significant decrease in Spontaneous Alternation (SA) compared to the control group.

Anxiolytic effect: In the elevated cross maze test, the number of times of lowering and the time spent in the open arms were used to evaluate the anxiolytic effect of the methanolic extract of *Eclipta alba* (Fig. 3). Thus, a significant decrease ($p<0.05$) in the number of times lowering and the time spent in the open arms was recorded in the group of methanolic extract of *Eclipta alba* (E.a+Sco) at 50 mg kg^{-1} (p.c) compared to the control group.

Antidepressant effect: When rodents were forced to swim in a confined space, after an initial period of struggle, they became immobile, resembling a state of mental despair and depression[15]. After being treated with the methanolic extract of *Eclipta alba* (E.a+Sco), swimming time increased non-significantly ($p>0.05$) compared to the control group (Fig. 4).

DISCUSSION

The methanolic extract of *Eclipta alba* shows a good content of polyphenolic compounds (18.85±0.61 mg EAG/100 mg and 6.38±1.05 mg EQ/100 mg). The total phenolic content of *Eclipta alba* (L.) Hassk., was much higher (0.30 mg GAE/100 mg extract) than that obtained for the same plant and the same type of extract by Surveswaran *et al.*[21]. Note that this author conducted his experiments in Hong Kong. This large difference could be explained by the fact that the synthesis of secondary metabolites by plants depends on their living environment. According to Kinda *et al.*[1], some flavonoids have anxiolytic effects and neuroprotective activities, the high content of total flavonoids in *Eclipta alba* could be responsible for the traditional use of this plant.

The evaluation of antioxidant activity by DPPH, ABTS and FRAP methods reveals that the methanolic extract of *Eclipta alba* has antioxidant activity. The antioxidant power of this extract was low compared to the references (quercetin and Trolox). This antioxidant power could be due to the presence of phenolic compounds and flavonoids. Indeed, polyphenols were highly hydroxylated compounds found in various fractions of plant extracts. One of the properties of polyphenols was their ability to neutralize free radicals in biological systems. This makes these compounds potential antioxidant agents[22].

For the acute toxicity study, all treated mice were closely examined for signs of toxicity from the time of extract administration until day 14 post extract administration. None showed obvious signs of toxicity or behavioural changes at 5000 mg kg^{-1} b.wt. All mice remained alive after the two weeks of observation. This showed that the LD_{50} was considered to be higher than 5000 mg kg^{-1} b.wt., by the oral route. The high LD_{50} value implies a low risk of acute poisoning and a high degree of relative safety[23,24] when the extract is administered orally. According to the OECD guideline, any drug or pharmaceutical compound with an oral LD_{50} greater than 2000 mg kg^{-1} could be considered safe or minimally toxic[25]. This result suggests that the methanolic extract of *Eclipta alba* is considered practically non-toxic and of high therapeutic index[26]. Current results corroborated previous studies reported that the aqueous extract of *Eclipta alba* is non-lethal by oral administration up to the dose of 2000 mg kg^{-1} b.wt.[27]. The weight gain was due to food and water intake and it could be said that the extract did not induce a loss of appetite during the period of the experiment.

At the end of the behavioural test, the percentage of spontaneous alternation of the group that received the methanolic extract of *Eclipta alba* decreased non-significantly compared to the control group. We can say that the methanolic extract of *Eclipta alba* could protect the memory against scopolamine-induced disorders. For the evaluation of anxiety, we have a significant decrease ($p<0.05$) in the number of times of lowering as well as the time spent in the open arms. This suggests that the methanolic extract of *Eclipta alba* did not have an anxiolytic effect at this dose. Regarding the antidepressant effect, the swimming time increased non-significantly ($p>0.05$) compared to the control group. This suggests that the methanolic extract of *Eclipta alba* (50 mg kg^{-1} b.wt.) at this dose could have an antidepressant-like effect. Our results corroborate previous studies that reported that *Eclipta alba* leaf extract (200 mg kg^{-1}) produced an antidepressant effect[28]. This study implies that the oral consumption of a dose of more than 5000 mg kg^{-1} would not cause any health problems, since it was found to be non-toxic. This constitutes a basis for the future production of phytomedicines for low-income populations. The limitations of this study are the lack of studies on the specific animal model of neuropsychiatric disorders. For the rest of our work, it is recommended to research specific animal models of neuropsychiatric disorders to arrive at more specific phytomedicines.

CONCLUSION

The phytochemical investigations have allowed highlighting the polyphenolic compounds. Thus the presence of these specific secondary metabolites could justify the use of *E. alba* in the traditional treatment of various diseases. The very low toxicity added to the antioxidant power of the plant brings safety to its traditional use in oral and anal ways. A current study has shown that extract of *E. alba* has pharmacological effects and justifies its use in the traditional medicine of Burkina Faso for the treatment of some neuropsychiatric disorders.

SIGNIFICANCE STATEMENT

This study discovered the reason for the consumption of an unquantified amount of *Eclipta alba* extract in traditional medicine without reported signs of toxicity. This is due to its high mean lethal dose (LD_{50}) signifying a high degree of relative safety that may be beneficial in the treatment of neuropsychiatric disorders. This study justified its use in traditional medicine in Burkina Faso.

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