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Qualitative Phytochemical Screening of Some Organs of *Pseudocedrela kotschyi* from the Togolese Flora and Comparative Study of their Anti-Diarrheal Activity

Anoumou Kodjo[1*], Novidzro Kosi Mawuéna[1], Mélila Mamatchi[1], Afankoutse Kokou Pierre[2], Awili Tètouwalla[3], Antena Manawa[2], Koumaglo Kossi Honoré[1]

[1] : Laboratory of Process Engineering and Natural Resources (LAGEPREN), University of Lomé (Togo)
[2] : Department of Animal Physiology, Faculty of Sciences, University of Lomé (Togo)
[3]: Department of Pharmacy, Faculty of Health Sciences, University of Lomé (Togo)
***Corresponding authour:** Anoumou Kodjo, Laboratory of Process Engineering and Natural Resources, Department of Chemistry, Faculty of Sciences, University of Lomé (Togo) ;
E-mail : vanoumou@yahoo.com

Abstract

The roots and trunk of *Pseudocedrela kotschyi* are commonly used to treat diarrhea, exposing this species to extinction. This study aims to compare the antidiarrheal activity of the leaves with that of the trunk and root bark for the preservation of biodiversity. The extracts were thus obtained successively in hexane, dichloromethane and ethanol by maceration. Qualitative phytochemical screening was carried out using staining and/or precipitation methods. Fractionation of the dry ethanolic extracts was carried out by column chromatography coupled with thin layer chromatography. The antidiarrheal activity of the extracts and their fractions was evaluated using wistar rats that were treated with the extracts and their fractions before administration of castor oil, used to induce diarrhea. Pretreatment of rats with loperamide, extracts and their fractions showed an inhibition of diarrhea ranging from 21.97% with the

leaf extract to 73.33% with loperamide, which constitutes the most significant activity. However, the root bark extract made it possible to obtain an inhibition of 70.55%, the inhibition closest to that obtained with loperamide and the highest considering all the extracts and their fractions. This justifies the use of root bark instead of leaves. However, the leaves can be considered, whose activity was not negligible, for a use that preserves biodiversity.

Keywords: *P. kotschyi*; leaves, trunk and root bark; phytochemical composition; antidiarrheal activity

Introduction

Diarrhea, a common digestive disorder, is characterized by the emission of loose or liquid and frequent stools, leading to dehydration of the body following the loss of water and electrolytes (WHO, 2017). It represents a major public health problem in developing countries. Indeed, each year, diarrheal diseases cause approximately 1.8 billion deaths worldwide, 90% of which are children under five years old, living in developing countries (Cazaban et al., 2005). They represent the third leading cause of mortality from infectious diseases for all ages (WHO, 2011; Assogba et al., 2012). Means to minimize risks and find solutions to this common digestive disorder are becoming a necessity for populations. Among various methods of combating or treating diarrhea, natural substances occupy an important place. Indeed, medicinal plants, for example, constituting a reservoir of active ingredients capable of treating several pathologies (Rani et al., 1999) are widely used for traditional therapy including the treatment of diarrhea. In Togo, 60 to 80% of the population, especially rural, treats itself with medicinal plants (Batawila, 2005; Sari, 2006). The Togolese flora is full of a large number of these medicinal plants, among which *Pseudocedrela kotschyi* is distinguished. In traditional medicine, this plant is used for several purposes. In particular, the local population mainly uses the root and trunk of this plant in the treatment of diarrhea and dysentery. However, there is not enough scientific data to justify this biological activity of the plant in order to guide users. Furthermore, it is necessary, for biodiversity-friendly sampling, to evaluate the effectiveness of the leaves compared to the root and trunk bark in order to contribute to the preservation of the species. This study is therefore carried out with the aim of verifying the effectiveness of the biomolecules contained in the organs of this plant responsible for its antidiarrheal properties.

Materials and Methods

Material

Laboratory equipment

Castor oil was used to induce diarrhea in rats while loperamide suspension was used to prevent it.

Plant material

Pseudocedrela kotschyi was harvested in September 2021 in Wahala in the Haho prefecture (Plateaux region), Togo. The plant was identified under the number "TOGO15976" at the Botany and Plant Ecology Laboratory of the Faculty of Sciences of the University of Lomé. The leaf and the trunk and root bark were then dried at laboratory temperature (20 – 25 °C), crushed and stored away from light and humidity for subsequent analyses.

Animal material

For the evaluation of antidiarrheal activity, Wistar strain rats of both sexes, weighing between 97 and 133 g were used. These rats were acclimatized to the production conditions in the animal house of the Department of Animal Physiology of the University of Lomé.

Methods

Sample preparation

Preparation of total extracts

An amount of 40 g of each powder of leaf, trunk and root barks of *P. kotschyi* was macerated with

400 mL of solvents of increasing polarities (hexane – dichloromethane –ethanol96%). The macerates thus obtained were incubated at room temperature and continuously stirred for 72 hours. They were then filtered and the ethanolic filtrates obtained were evaporated using a rotary evaporator at 45 °C. The ethanolic residues obtained were stored at -23 °C for their further use.

Qualitative phytochemical characterizations of ethanolic extracts

The different families of biomolecules present in the ethanolic extracts of leaf and the trunk and root barks of *Pseudocedrela Kotschyi* were highlighted by the staining and/or precipitation tests. Indeed, the alkaloids were sought by the reaction exploiting the Dragendorff reagent(Jamal et al., 2024); polyphenols and tannins, respectively based on the Stiasny reagent and the test with $FeCl_3$(Devi et al., 2024); flavonoids, by reaction with cyanidin (Houta et al., 2012); saponins, thanks to the foam test (Devi et al., 2024); and finally sterols and triterpenes, by the Liebermann-Bürchard test(Karime et al., 2020).

Splitting

Fractionation of dry extracts was performed by column chromatography coupled with thin layer chromatography. Column chromatography was performed using a binary mixture of three solvents (hexane, ethyl acetate and methanol) as eluent and the stationary phase was silica gel. The mobile phase used for thin layer chromatography was a binary or ternary mixture consisting of hexane, ethyl acetate, methanol and acetic acid.

Induction of diarrhea by castor oil

All rats were orally administered 2 mL of castor oil according to the method described by Shoba and Thomas (2001) and Méité et al. (2009). Before treatment, rats were fasted for 24 hours and then 20 groups of rats were formed as follows:

- The first group of rats received distilled water (control group).

- The second group of animals received loperamide (0.2 mg/mL), the reference drug, at a dose of 15 ml/kg, 60 minutes before castor oil administration.

- All other groups were divided into two subgroups for administration of total extracts at doses of 100 and 250 mg/kg BW, 60 minutes before administration of castor oil and 25 and 50 mg/kg BW, 60 minutes before administration of castor oil for fractions.

Four hours after the induction of diarrhea, the faeces emitted by the rats of each group were carefully collected and weighed. The percentage of inhibition of diarrhea for each group of treated rats was determined by the following formula:

$$T = [(P_0 - P)/P_0] \times 100$$

T: Percentage of inhibition;
P_0: Average weight of faeces in the control group: group A.
P: Average weight of faeces in each treated group.

The frequency of emission and appearance of faeces were also assessed.

Statistical analysis

Data were entered using Microsoft Office Excel version 2019 spreadsheet and analyzed using Graph Pad Prism 8.0.2 software (263). Results were expressed as mean plus or minus standard error of the mean (M ± SEM).

Differences were considered significant at the 5% level ($p<0.05$).

Results

Phytochemical analysis of extracts

Table I: Results of phytochemical screening of the three organs of *P. kotschyi*

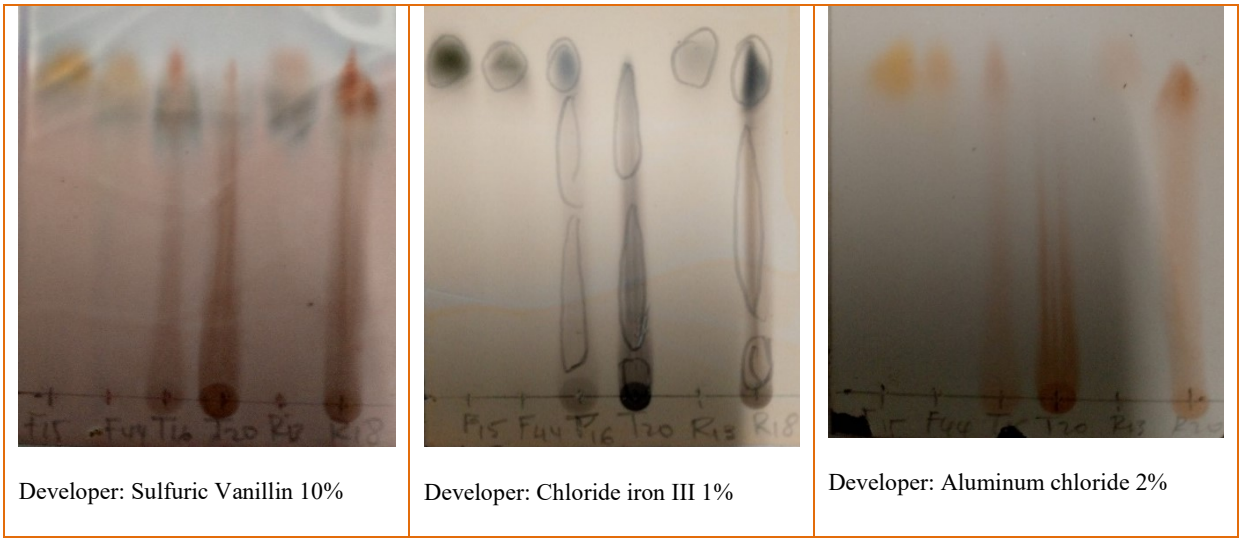
	Le-Ext	Tr-Ext	Ro-Ext
Alkaloids	+	+	+
Phenolics compounds	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Saponins	+	+	+
Triterpenes et sterols	-	-	-

Le-Ext: leaf extract; Tr-Ext: trunk bark extract; Ro-Ext: Root bark extract; +: Presence of the compound analyzed; -: Absence of the compound analyzed.

Splitting results

At the end of column chromatography coupled with thin-layer chromatography, 6 fractions, two of which were from each total extract, were obtained.

- R13: ethyl acetate-methanol fraction (8:2) of the ethanolic extract of the root bark;
- R18: ethyl acetate-methanol (6:4) fraction of the ethanolic extract of the root bark;
- FH15: ethyl acetate-methanol fraction (5:5) of the ethanolic extract of the leaves;
- F44: ethyl acetate-methanol fraction (4:6) of the ethanolic extract of the leaves
- T16: ethyl acetate-methanol fraction (8:2) of the ethanolic extract of the trunk bark;
- T20: ethyl acetate-methanol fraction (5:5) of the ethanolic extract of the trunk bark.



Developer: Sulfuric Vanillin 10% | Developer: Chloride iron III 1% | Developer: Aluminum chloride 2%

Figure 1: Thin Layer Chromatography (TLC) results of the fractions

Chemical screening on TLC plate demonstrated the presence of the compounds in all fractions. Fractions FH15 and F44 appear to have a similar profile. 10% sulfuric vanillin shows that all fractions contain several compounds. In general, specific developers prove that all fractions contain identical compounds. The use of a universal developer (sulfuric vanillin) followed by chlorideiron III 1% indicating the presence of polyphenols and tannins and aluminum chloride which revealed the presence of flavonoids shows that all six fractions would contain phenolic compounds, tannins and especially flavonoids in high concentrations (Lahadson, 2016).

Anti-diarrheal effect

Table 1: Results of antidiarrheal activity of extracts and fractions of *P. kotschyi* after four hours

Sample	Frequency of faecal emissionafter 4 hours	Total faecal weight (g)after 4 hours	Percent of inhibition (%)
Eau	04.00 ± 0.41	03.67 ± 0.14	00.00
LPA (3 mg/Kg PC)	00.75 ± 0.25	0.98 ± 0.28**	73.33
Ro-Ext (100 mg/Kg PC)	01.75 ± 0.25	02.13 ± 0.20**	42.06
Ro-Ext (250 mg/Kg PC)	01.00 ± 0.00	01.08 ± 0.14**	70.55
R13 (25 mg/Kg PC)	02.5 ± 0.29	02.72 ± 0.21**	25.84
R13 (50 mg/Kg PC)	01.5 ± 0.29	02.75 ± 0.41**	25.02
R18 (25 mg/Kg PC)	01.75 ± 0.25	01.51 ± 0.21***	58.88
R18 (50 mg/Kg PC)	01.75 ± 0.48	01.52 ± 0.14**	58.62
Tr-Ext (100 mg/Kg PC)	02.25 ± 0.25	02.55 ± 0.26**	30.41
Tr-Ext (250 mg/Kg PC)	01.75 ± 0.25	01.84 ± 0.05**	49.83
T16 (25 mg/Kg PC)	02.00 ± 0.00	02.57 ± 0.21**	29.93
T16 (50 mg/Kg PC)	01.75 ± 0.48	01.63 ± 0.15**	55.50
T20 (25 mg/Kg PC)	01.75 ± 0.25	01.95 ± 0.22**	46.75
T20 (50 mg/Kg PC)	01.75 ± 0.25	01.63 ± 0.15**	55.50
Le-Ext (100 mg/Kg PC)	02.00 ± 0.00	02.86 ± 0.10**	21.97
Le-Ext (250 mg/Kg PC)	02.00 ± 0.00	02.67 ± 0.08**	27.21
FH15 (25 mg/Kg PC)	02.00 ± 0.00	02.55 ± 0.28	30.47
FH15 (50 mg/Kg PC)	01.75 ± 0.25	02.52 ± 0.21**	31.29
F44 (25 mg/Kg PC)	02.25 ± 0.25	02.25 ± 0.14**	38.57
F44 (50 mg/Kg PC)	01.75 ± 0.25	01.87 ± 0.20**	49.83

Results are expressed as mean ± SEM; n = 4; ** $p < 0.01$; *** $p < 0.001$ compared to control (water). LPA: Loperamide;Ro-Ext: Root bark extract; Tr-Ext: Trunk bark extract; Le-Ext: Leaf extract; R13: AcEt – Met fraction (8:2) of root extract; R18: AcEt – Met fraction (6:4) of root extract; T16: AcEt – Met fraction (8:2) of trunk extract; T20: AcEt – Met fraction (5:5) of trunk extract; FH15: AcEt – Met fraction (5:5) of trunk extract; F44: AcEt - Met fraction (4:6) of trunk extract PC: Body weight; AcEt: Ethyl acetate; Met: Methanol The data highlighted in yellow are those obtained with the positive (treatment with loperamide) and negative (treatment with distilled water) controls, the data highlighted in red are those obtained with the total extracts and the non-highlighted data are those obtained with the fractions of extracts considered.

It reveals that after 4 hours, the ethanolic extracts of the root and the trunk barks and the leaf, their different fractions or loperamide decrease the quantity and frequency of faeces of rats (Table 1). Indeed, the weight of faeces of the diarrheic control rats is 03.67 ± 0.14 g. However, by treating the rats on the one hand, with the extracts of *P. Kotschyi* at doses of 100 and 250 mg/Kg BW and on the other hand, with the fractions of these extracts at doses of 25 and 50 mg/Kg BW,

the weight of faeces decreased in a dose-dependent manner. The weight of the rats' faeces, first for the root bark extract, were 2.13 ± 0.20 g and 1.08 ± 0.14 g, i.e. respective reductions of 42.06% and 70.55% ($p < 0.01$). Then, for the trunk bark extract, these weights were 2.55 ± 0.26 g and 1.84 ± 0.05 g, i.e. respective reductions of 30.41% and 49.83% ($p < 0.01$). Finally, for the leaf extract the weights were 2.86 ± 0.10 g and 2.67 ± 0.08 g, i.e. respective reductions of 21.97% and 27.21% ($p < 0.01$) respectively at doses of 100 and 250 mg/Kg PC. For the root bark fractions, the faecalweight were respectively 2.72 ± 0.21 g and 2.75 ± 0.41 g, i.e. respective reductions of 25.84% and 25.02% ($p < 0.01$) for R13 on the one hand and on the other hand, 1.51 ± 0.21 g and 1.52 ± 0.14 g, i.e. respective reductions of 58.88% ($p < 0.001$) and 58.62% ($p < 0.01$) for R18 at doses of 25 and 50 mg/kg BW. With the fractions of the trunk bark, the weight of the faeces at doses of 25 and 50 mg/Kg BW were respectively 2.57 ± 0.25 g and 1.63 ± 0.15 g, i.e. respective reductions of 29.93% and 55.5% ($p < 0.01$) for T16 on the one hand and on the other hand, 1.95 ± 0.22 g and 1.63 ± 0.17 g, i.e. respective reductions of 46.75% and 55.5% ($p < 0.01$) for T20. At these same doses, the fractions of the leaf extract, for FH15, the weight of faeces were 2.55 ± 0.28 g and 2.52 ± 0.21 g, i.e. respective reductions of 30.47% and 31.29% ($p < 0.01$) then for F44, these weightswere 2.25 ± 0.14 g and 1.87 ± 0.20 g, i.e. respective reductions of 38.57% and 49.83% ($p < 0.01$). Furthermore, the frequency of faeces of a diarrheic control rat was 4 times; that of the samples on average was approximately 2 times whatever the dose. The extract of the root bark was more antidiarrheal with a single emission per rat. For rats treated with loperamides at 3 mg/kg BW before receiving castor oil, the faecal weight was 0.98 ± 0.28 g. As for the appearance of the stools, watery stools were more noticeable in rats treated with the leaf extract and its fractions. The reduction is also very significant at 73.33% ($p < 0.01$) with approximately one emission per rat. The reductions are compared with diarrheic control rats. The frequency of faecal passage was approximately two times per rat for all samples. Liquid and semi-liquid faecal were observed in all rats except those given the ethanolic extract of the root barks at a dose of250 mg/kg BW and loperamide.

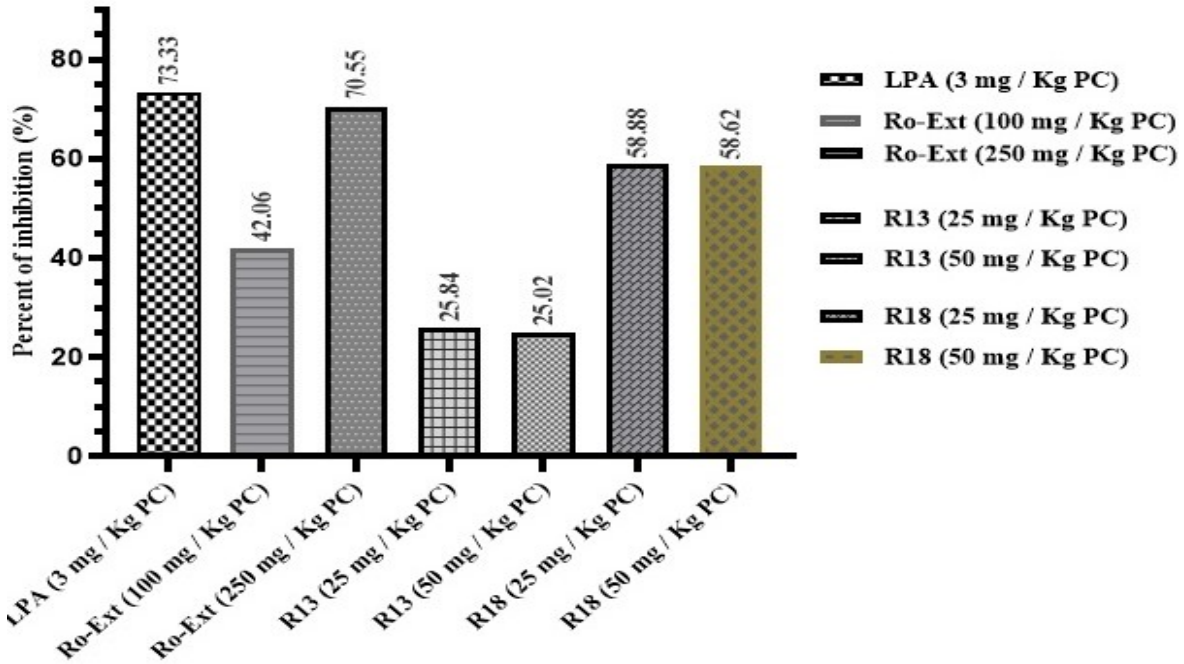


Figure 2: Inhibition rate of diarrhea in rats treated with castor oil by ethanolic extract of root bark, its fractions and loperamide after 4 hours

LPA: Loperamide; Ro-Ext: Ethanolic root extract; R13: AcEt – Met fraction (8:2) of root extract; R18: AcEt – Met fraction (6:4) of root extract; PC: Body weight; AcEt: Ethyl acetate; Met: Methanol

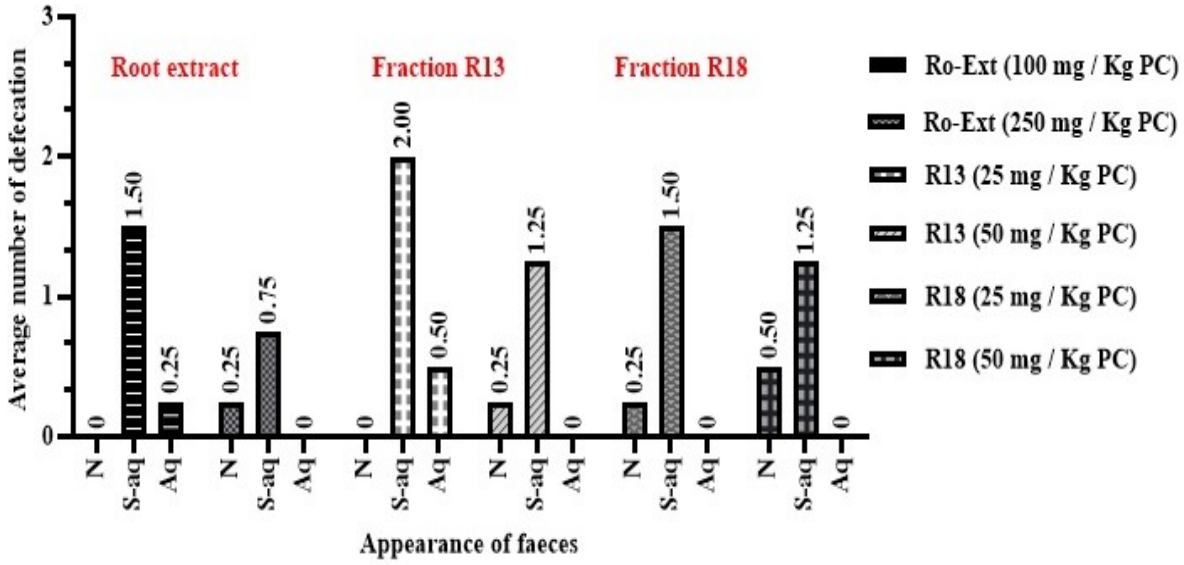


Figure 3: Effects of ethanolic extract of root bark and its fractions on the number and appearance of faeces of a rat treated with castor oil after 4 hours

Ro-Ext: Ethanolic root extract; R13: AcEt–Met fraction (8:2) of root extract; R18: AcEt–Met fraction (6:4) of root extract; PC: Body weight; AcEt: Ethyl acetate; Met: Methanol, N: Normal; S-aq: Semi-aqueous; Aq: Aqueous.

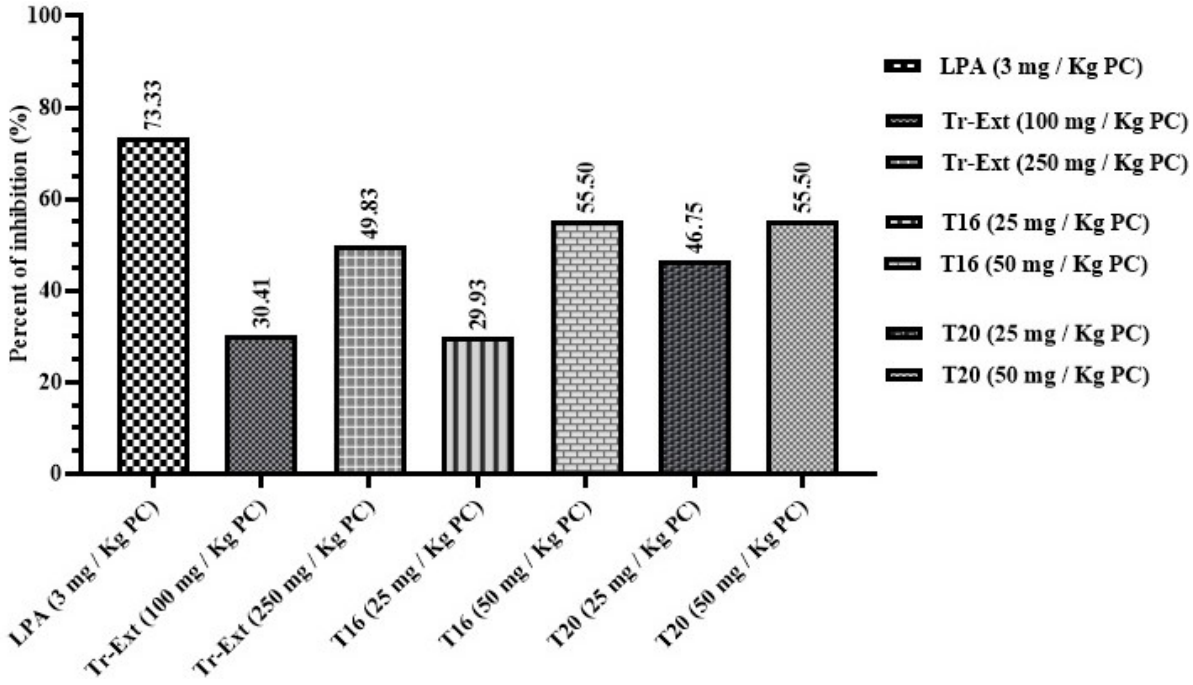


Figure 4: Inhibition rate of diarrhea in rats treated with castor oil by ethanolic extract of trunk bark, its fractions and loperamide after 4 hours

LPA: Loperamide; Tr-Ext: Ethanolic extract of the trunk; T16: AcEt – Met fraction (8:2) of the trunk extract; T20: AcEt – Met fraction (5:5) of the trunk extract; PC: Body weight; AcEt: Ethyl acetate; Met: Methanol

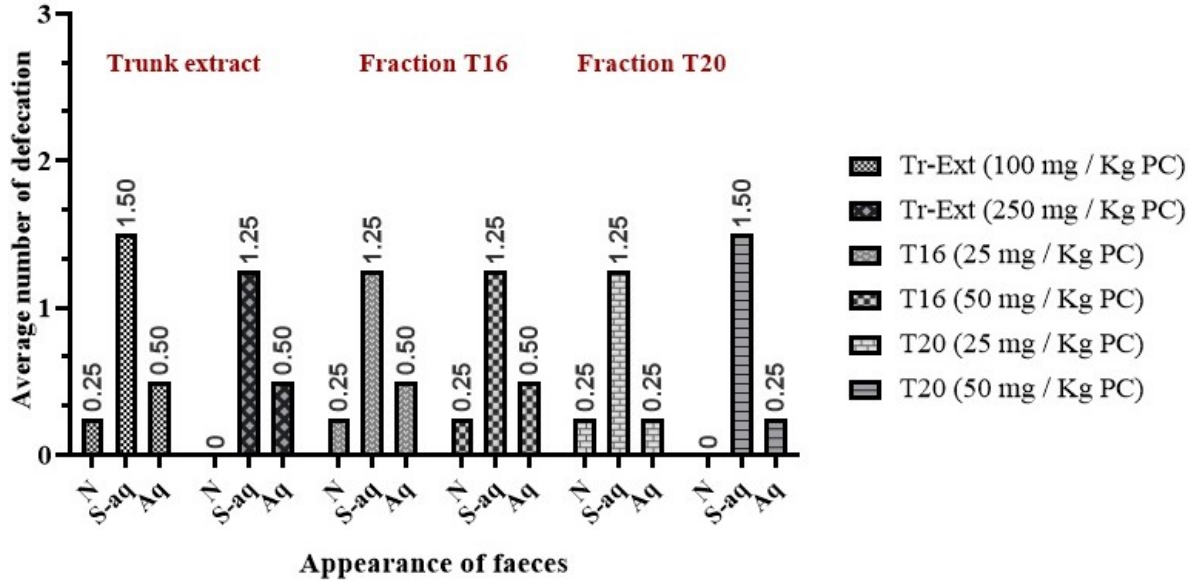


Figure 5: Effects of ethanolic extract of trunk bark and its fractions on the number and appearance of faeces of a rat treated with castor oil after 4 hours

Tr-Ext: Ethanolic extract of the trunk; T16: AcEt – Met fraction (8:2) of the trunk extract; T20: AcEt – Met fraction (5:5) of the trunk extract; PC: Body weight; AcEt: Ethyl acetate; Met: Methanol; N: Normal; S-aq: Semi-aqueous; Aq: Aqueous.

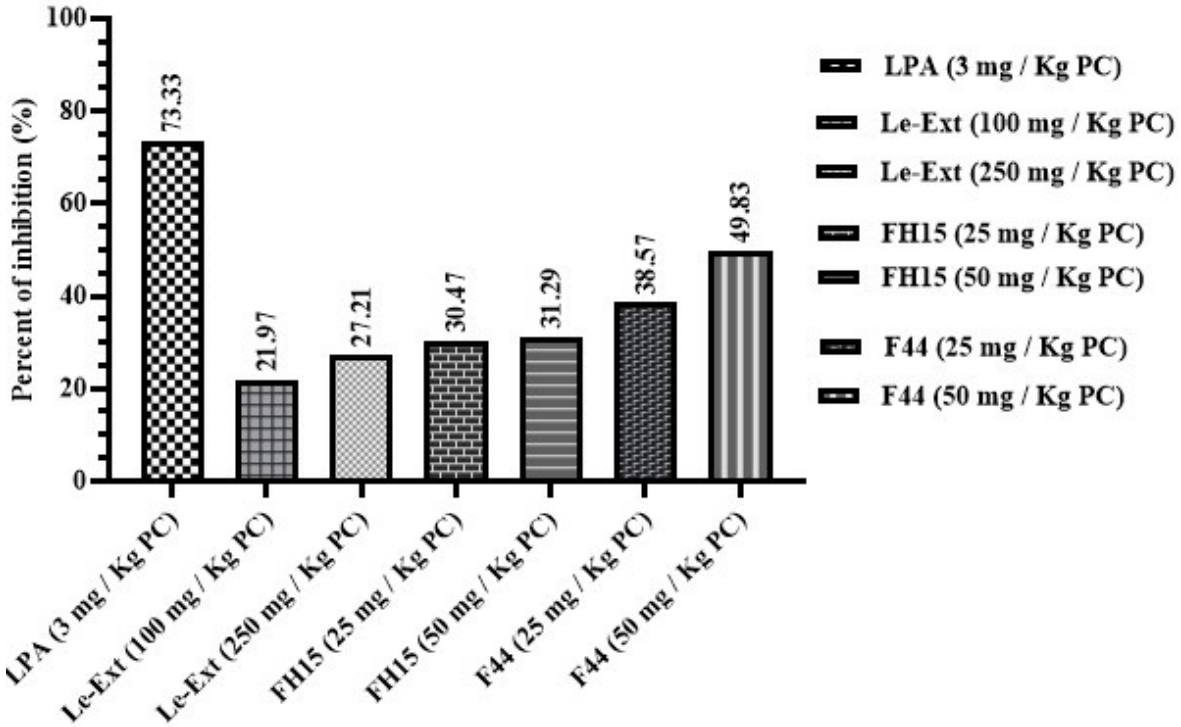


Figure 6: Inhibition rate of diarrhea in rats treated with castor oil by ethanolic extract of leaves, its fractions and loperamide after 4 hours

LPA: Loperamide; Le-Ext: Ethanolic extract of the leaf; FH15: AcEt – Met fraction (5:5) of the trunk extract; F44: AcEt - Met fraction (4:6) of the trunk extract; PC: Body weight; AcEt: Ethyl acetate; Met: Methanol

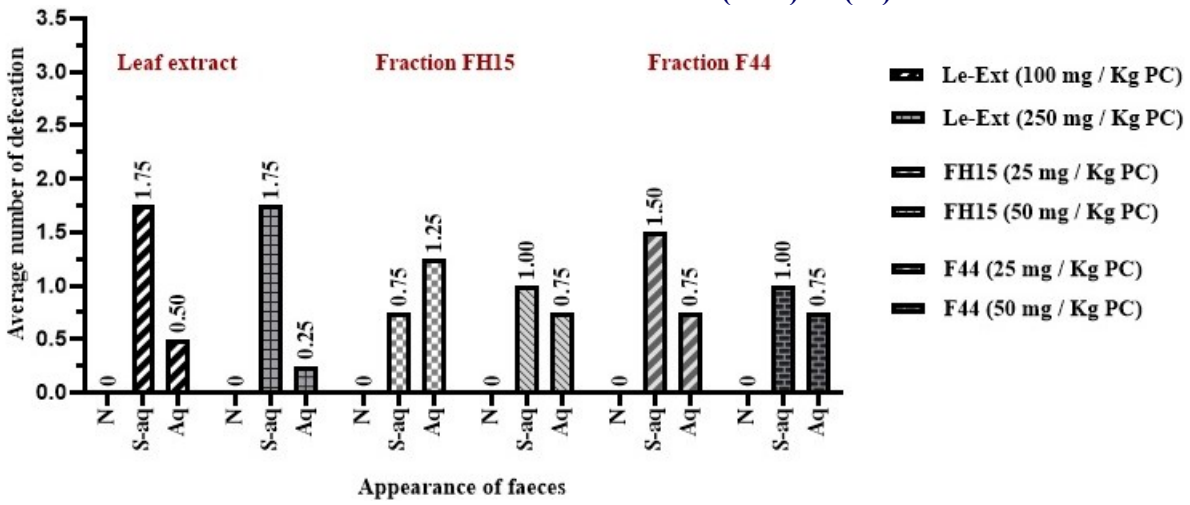


Figure 7: Effect of the ethanolic extract of the leaf and its fractions on the number and appearance of faeces of a rat treated with castor oil after 4 hours

LPA: Loperamide; Le-Ext: Ethanolic extract of the leaf; FH15: AcEt – Met fraction (5:5) of the trunk extract; F44: AcEt - Met fraction (4:6) of the trunk extract; PC: Body weight; AcEt: Ethyl acetate; Met: Methanol; N: Normal; S-aq: Semi-aqueous, Aq: Aqueous.

Discussion

The antidiarrheal activity of total extracts and fractions of some organs of *P. kotschyi* was evaluated through this study. Phytochemical tests carried out on the considered extracts revealed the presence of tannins, flavonoids, polyphenols, saponins and alkaloids in the leaves and barks of the trunk and rootof *P. kotschyi.* However, triterpenes and sterols were not revealed in the analyzed extracts. These results are similar to those reported with leaves (Akuodor et al., 2013; Essiet et al., 2016), trunk barks (Adeniyi et al., 2010; Alhassan et al., 2014) and root barks (Bothon et al., 2013; Ojewale et al., 2013). However, sterols and triperpenes were not revealed in this study.

Column chromatography coupled with thin layer chromatography performed on the extracts for fractionation with the developers used on the 6 fractions obtained shows that the latter are rich in phenolic compounds, tannins and especially flavonoids. Indeed, flavonoids, polyphenols, tannins and saponins are known for several biological activities including antidiarrheal activity (Sérémé et al., 2008; Dosso et al., 2012; Ambe et al., 2015)

To study the antidiarrheal activity, diarrhea was induced in the present study by castor oil. Indeed,Castor oil causes diarrhea due to its active component, ricinolic acid which increases peristaltic activity in the small intestine, leading to the alteration of electrolytic permeability of the intestinal mucosa (Akuodor et al., 2010).Total ethanolic extracts of leaves, trunk barks and root administered to rats at doses of 100 and 250 mg/kg BW before treatment with castor oil, resulted in a significant decrease ($p < 0.01$) in faecalweight and a delay in the appearance of these faecal matter in a dose-dependent manner. The inhibition was more noticeable in rats treated with the ethanolic extract of *P. kotschyi* root barks at a dose of 250 mg/kg BW without aqueous faeces. The lowest diarrhoeal inhibition was recorded in rats that received the ethanolic extract of the leaves of this species at a dose of 250 mg/kg BW with higher aqueous faeces preceded by that of the extract of the trunk barks at the same dose. These results are consistent with those reported by Bennini and Merdaci (2016) with the aqueous extract of Nigella Sativa seeds at doses of 250 and 500 mg/kg BW causing a significant decrease in faecalweight in treated diarrheic rats. Furthermore, Chitme et al. (2004), Atta and Mouneir (2005) and N'guessan et al. (2020) showed that in rats given castor oil after

pretreatment with, respectively, the ethanolic extract of Calotropis gigantea leaves at doses of 200 and 400 mg/kg BW, the ethyl acetate extract of Bidens bipinnata leaves at doses of 200 and 400 mg/kg BW and the aqueous extract of Solanum Torvum at doses of 150 and 250 mg/kg BW, the frequency of faeces was reduced as well as a significant decrease in diarrheal droppings.

As for the fractions, the inhibition rates of the fractions of the ethanolic extract of the leaves, although they are lower than those of the fractions of the other extracts at the same dose of 50 mg/kg BW, are appreciable. Despite the low dose (50 mg/kg BW) of the fractions of the ethanolic extract of the leaves (250 mg/kg BW), these fractions were more inhibitory of diarrhea than the crude extract. The same is true for the ethanolic extract of the bark of the trunk (250 mg/kg BW) and its fractions (50 mg/kg BW). This could be explained by the presence of certain compounds in these extracts which have an antagonistic effect on the biomolecules present in the fractions(Popovici et al., 2009).

The inhibitory effects of *P. kotschyi* extracts and their fractions on faecalweight and intestinal transit time are also obtained with loperamide. It acts selectively on the intestine by stimulating the absorption of water and electrolytes at the enterocyte level and inhibits peristalsis by inhibiting calmodulin, thus increasing the transit time of the contents of the digestive tract (N'guessan et al., 2020). The samples (extracts and fractions) at their different doses would thus have a mechanism of action similar to that of loperamide (the reference antidiarrheal) at 3 mg/kg BW. The ethanolic extract of *P. kotschyi* root bark at a dose of 250 mg/kg BW has an effect very close to this reference antidiarrheal. The decrease in faecalweight and the slowing down of intestinal transit noted in rats pretreated with the extracts and their fractions would certainly be due to the presence of tannins, flavonoids, polyphenols and saponins known for their antidiarrheal properties and as revealed by the phytochemical screening of the extracts considered (Sérémé et al., 2008). In the present study, the results obtained in favor of root compared to trunk barks and leaves justify the users' choice for root barks during sampling. However, even if the ethanolic extract of the leaves showed a relatively low inhibitory effect on diarrhea at a dose of 250 mg/kg BW, its non-toxic effect (Kabiru et al., 2015, Ezeokpo et al., 2020) and the use of the leaves having little impact on the sustainability of this species require their collection compared to the root and trunk barks which alter it more. Furthermore, Essiet et al. (2016) reported that the ethanolic extract of the leaves inhibits diarrhea by up to 91%. This shows that the antidiarrheal activity of these leaves is not negligible and can be considered at a higher dose for more effective activity.

Conclusion

The study of the antidiarrheal activityin vivo of ethanolic extracts of the leaf, trunk and rootbark of *P. kotschyi* and their fractions has shown that this plant has antidiarrheal properties that could be explained by its richness in phytochemical compounds. Diarrhea, induced by castor oil in rats was indeed slowed down by ethanolic extracts of the three organs of the plant and their fractions with a more effective activity for the root. Metabolites such as flavonoids, tannins, polyphenols and saponins revealed by phytochemical screening would be, among others, at the origin of this activity. Overall, even if the results obtained show that the root bark and its fractions have a more interesting antidiarrheal activity, we can also consider the leaves whose activity was not negligible. This would allow a use that preserves the species and therefore respects biodiversity.

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