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SCREENING AND ANTHELMINTIC ACTIVITY OF TWO MEDICINAL PLANTS *MYRICA SALICIFOLIA* AND *PSYCHOTRIA PALUSTRIS* CONSUMED BY APES FOR AUTOMEDICATION IN KAHUZI-BIEGA NATIONAL PARK

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ABSTRACT

In Kahuzi-Biega National Park, great apes are frequently affected by helminthic diseases as observed in their dungs. To understand the auto-medication mechanisms of two plants ingested by great apes (*Myrica salicifolia* and *Psychotria palustris)* is necessary to evaluate bioactive proprieties and control worms. Phytochemical screening, flavonoids extraction and fractioning was done according to standards methods. In vitro anthelminthic activity against *Alma emini* was used to evaluate the potential of the two plants. Positive control was compared with mebendazole and albendazole solutions prepared and while negative control was deionized water. *Myrica salicifolia* has high anthelmintic activity and flavoinoids separated in the plants revealed two marks which have different position. Lethal concentration varied from plants species and extracted solvent (ethanol /or water). Aqueous extract has lower lethal concentration compared to ethanolic extract. The aqueous concentration is active than ethanolic ones ($p< 0.05$).The concentration of 1 mg/mL of flavonoids for fraction 1 is active to *Alma emini* in vitro. The concentration of 0.1 mg/mL is not activeto *Alma emini*. The findings from the current study revealed that extracts from *Myrica salicifolia* and *Psychotria palustris* have shown promising *in vitro* anthelmintic activity against *Alma emini* earthworm. Detailed analyses are needed by using different active constituents contained in the plants in order to assess their therapeutic role in different species of parasites infecting wild animals in the park.

KEYWORDS: Phytochemical screening, anthelmintic activity, Ape foods, auto-medication.

INTRODUCTION

The World Health Organization estimates that a staggering two billion people harbor parasitic worm infections. Helminth parasites are a major problem in animals throughout the world resulting in significant production losses as the performance of animals is inversely correlated with the intensity of helminth infections (Tariq and Tantry, 2012).The great apes are naturally susceptible to many diseases including parasites that afflict humans and great apes (Masi et al., 2012).Parasitic worms also infect apes in forest and have negative impact on animals in a conservation area. Great apes are known to intentionally consume plants with low nutritional value and/or high bioactive compounds possibly to maintain and improve their health suggesting a self-medicating role (Huffman and Seifu, 1989; Kamungu et al., 2015a). A variety of non-nutritional plant secondary metabolites are found in the great ape diet, but little is known about the possible medicinal consequences of their ingestion. One of the challenges of interpreting self- medication in animals is to distinguish between possible indirect medicinal benefits derived from secondary metabolites rich in plants that are assumed to be ingested for their nutritional value versus limited situation when specific ingestion of items are processed solely for their curative value or other physiological effects. Certain toxic plants are beneficial for health if small amounts are ingested infrequently and in a specific context of illness. Great apes have been found to consume plants with pharmacological properties (Krief et al, 2006). Indeed, phytochemical analysis of such plants has revealed the presence of bioactive compounds effective against systemic and intestinal parasites (Jisaka et al., 1993; Huffman, 2003; Krief et al., 2005; Krief et al., 2006).

A lot of work has been done in the field of plant eaten by great apes including both ethnobotanical surveys and scientific validation of their toxic efficacies in many parts of the world (Jisaka et al., 1993; Huffman,

2003;Krief et al., 2005; Kamungu et al., 2015a; Kamungu et al., 2015b). However, little is known about auto-medication role of plants consumed by the great apesinhabiting Kahuzi/Biega National Park. This paper presents a comparative study to evaluate bioactive proprieties between two plants ingested by great apes in Kahuzi-Biega National Park (*Myrica salicifolia* and *Psychotria palustris)* to understand the auto-medication mechanisms of the great apes to control helminthic diseases since worms are frequently observed in their dungs.

MATERIAL AND METHODS

2.1. Collection of plants and preparation of the extract

The two plant samples showed larvicidal activity to mosquito larvae which is manifested by a high percentage of mortality in comparison to those in the control group (Bagalwa et al., 2017). *Myrica salicifolia* and *Psychotria palustris* have been founded in the list plants species eaten by apes at Kahuzi National Park (Basabose, 2002; Yamagiwa, 2009).

Barks of *Myrica salicifolia* and *Psychotria palustris* were collected in Kahuzi-Biega National Park. Voucher specimens of each plant are conserved in the herbarium of the "Centre de Recherche en Sciences Naturelles" of Lwiro for further references. The plants materials were then shade dried for one month. After complete drying the plants materials was porously powdered mechanically and was subjected to cold extraction using maceration process with water and ethanol as the solvents system for 24 h. After the extraction, water and ethanol containing plant bioactive compounds were filtered and concentrated in a rotavapor allowing a complete evaporation of the solvent. Separated ethanolic and aqueous extracts diluted at different concentrations were prepared for further phytochemicals screening tests.

2.2. Phytochemical analysis

To detect the presence of phenols, tannins, alkaloids, flavonoids, steroids, terpenes, saponins and alkaloids several phytochemical tests were performed following the method described by Chifundera et al. (1993). These tests are based on visual observation of color modification or precipitate formation after mixing specific reagents.

To detect presence of *Alkaloids* 5 mL of aqueous extract was introducing in 4 tubes each. Mayer's reagent was added to one tube, Wagner's reagent in a second, Erddtman's reagent in the third and Draggendoff's reagent in forth tube. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggendoff's and Wagner's reagents) or green, yellow coloration (Erddtman's reagent) was regarded as positive for the presence of alkaloids.

As for Saponins 5 mL of distilled water was added in a test tube. The solution was shaken vigorously byhand and observed for the presence of a stable persistent froth. The resultant frothing was mixed with three drops of olive oil and shaken vigorously. The appearance of creamy mass of small bubbles indicated the presence of saponins.

Occurrence of *Tannins* was testedin mixing 5 mL of aqueous extract with a few drops of 0.1 per cent ferric chloride. The presence of tannins was confirmed after observing appearance of brownish green or a blue-black coloration.

For *Flavonoids* about 0.5 g of the plant extracts was dissolved in diluted sodium hydroxide and hydrochloric acid and was added to the solution. A yellow solution that later turns a colorless solution indicated the presence of flavonoids.

Presence of *Steroids* was confirmed by adding 2 ml acetic anhydride to 5 mL of the ethanolic extract along with 2ml sulphuric acid. If the color changes from violet to blue or green in it indicates the presence of steroids.

Lastly, presence of *Terpenoids* (Salkowski method) was tested by mixing 5 mL of the ethanolic extract with 2 mL of chloroform in a test tube. Concentrated sulphuric acid (3 ml) was carefully added to the same test tube to form a layer. Positive tests confirming the presence of terpenoids was indicated by a reddish brown coloration of the interface.

2.3. Preparation of dilutions

The dried extracted residue containing the active ingredients previously weighed, were taken up in 5 ml of deionized water. Various concentrations were made (1/10em) with deionized water. The aqueous extracts were diluted to concentrations of 100, 10, 5, 1, 0.5, and 0.1 mg/ml in a total volume of 5 ml while the ethanolic extracts were diluted to concentrations of 400, 20, 4, 2, 0.4and 0.2 mg/ml in a total volume of 5 ml.

The positive control solutions were separately formed by crushing 100 mg of mebendazole tablet (100 mg) and albendazole (400 mg) tablet using a mortar to obtain fine powders. The powder for each tablet was then dissolved into 100 ml of distilled water to obtain solutions of mebendazole (1mg/ml), decaris (5mg/ml) and that of albendazole (4mg/ml),respectively.

Whereas distilled water were served as negative control.

2.4. Flavonoids extraction

Ethanolic extract obtainedwas dissolved in 20 mL of boiled water and keep in the laboratory at room temperature for 24 h. The solution was mix respectively with 40 mL of ether, 40 mL of ethyl acetate and 50 mL of n-butanol respectively (Djarhra et al., 2012).The obtained solution was again evaporated and the crude was keep for chromatography identification and separation and for helminthic test.

2.5. Flavonoïds separation

A sample of flavonoids crude obtained was submitted to separation by chromatography silica gel sheets (Silica gel 60 F_{254} TLC plates) and developed in ethyl acetate, methanol and water (5-2-1). Another chromatography silica gel sheet was eluted by the mixed solvent butanal, acetic acid and water (4-1-5). Plates for the first elute were sprayed with aluminium chloride ($AlCl_3$) at 2 % mixed in methanol 95 % (Ngbolua et al., 2014). Plate eluted in BAW was developed with active Iodate. The active constituents were detected as yellow, yellow green or spots on ochrebackground (Donzo et al., 2015). Only zones where their color turned from violet to yellow within the first 30 min (after spraying) were taken as positive results. The number of the type of flavonoid was none and was then separate by column chromatography using the same eluant of mixing acetate, methanol and water. The separated solution was evaporated and test for helminthic activity.

2.6. In vitro anthelmintic activity test of the extract

This test was conducted using alive earthworms. Earthworm was selected as a model for anthelmintic activity due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings (Thorn et al., 1977; Vigar, 1984; Nisha et al., 2012). Adult alive earthworms (*Alma emini)* collected in the wetland from moist soil near the "Centre de Recherche en Sciences Naturelles de Lwiro" in DR Congo were maintained under normal mud with water, for about two or four days. Before the experiment earthworms were washed with water. All alive adult earthworms used for the experiment were of approximately same sizes (4 cm in length and 0.2 - 0.3 cmin width). *Alma emini*, an oligochaete glossoscolecid worm that was used for bioassay in this study was already used a previous similar study for evaluation of anthelmintic activities of *Viscum congolese* and *Galiniera confeoides* (Bahizire et al., 2015).

The anthelmintic activity was evaluated as per the method of Dash et al. (2002), slightly modified (Vigar, 1984). Six alive earthworm individuals were released into 10 ml of desired concentration of plant extract, water and synthetic products such as decaris, mebendanzole and albendazole (Singh et al., 2011; Sharma et al., 2011). The earthworms were deposedin different prepared concentrations of the two plants, positive and negative control solutions for 24 hours. Death was concluded based on total loss of motility with faded body color. Each test was repeated at least three times. Mortality rates was computerized to give the lethal concentration (LC) determined by probit statistical curves analysis.

2.7. Statistical analysis

Data from experiment were transformed by probit transformation against the logarithm of extract concentration. The extract concentration required to cause50% (CL_{50}) mortality of earthworms was calculated using probit analysis. Comparison of mean percentages of mortality of *Alma emini* earthworm at different concentrations with the positive and negative control was performed by one way ANOVA.

RESULTS

A phytochemical screening was realized and the main secondary metabolites found in the two tested plants are presented in the table 1.

Table 1: Major classes of secondary metabolites found in the *Myrica salicifolia* and *Psychotria palustris.*

	Myrica salicifolia	***Psychotria palustris***
Alkaloids	+++	+++
Carotenoids	++	+++
Terpenoids	++	+++
Steroids	+++	++
Tannins	+++	+++
Saponines	+++	+++
Quinones	+++	+++
Phenols	+++	+++
Lipoids	+++	+++
Glycosides	++	+++
Flavonoids	++	+++

Legend: ++ = positive reaction (presence); +++ = strong positive reaction (strong presence)

Most of the plants have major secondary metabolites like flavonoids, glycosides, steroids, alkaloids, phenolic compounds, saponines, quinones and tannins. *Psychotria palustris* has high number of secondary components with strong positive anthelminthic reaction. Saponin, alkaloids, tannins, quinones, phenols, lipoids were detected in both tested plants whereas steroids were particularly present in high concentration in *Myrica salicifolia* while terpenoids, carotenoids, glycosides and flavonoids were strongly present in *Psychotria palustris.*

The results of anthelmintic activity are presented in table 2.

Table 2. Anthelmintic activity of *Myrica salicifolia* and *Psychotria palustris* against *Alma* emini (12 in exposition).

Plants	Extracts	Concentration (mg/L)	Meanmortality	Pourcentage mortality (%)
Myrica salicifolia	Aqueous	0.5	0	0
		1	9	75
		5	12	100
	Ethanolic	0.2	0	0
		0.4	1	8.3
		2	4	33.33
		4	8	66.66
		20	12	100
Psychotria palustris	Aqueous	1	0	0
		5	4	33.33
		10	12	100
	Ethanolic	4	0	0
		20	4	33.33
		40	12	100
Decaris		5	12	100
Mebendanzole		1	12	100
Albendazole		4	12	100
Water (control)			0	0

Lethal concentration varied from plants species and extracted solvent (ethanol /or water). Aqueous extract has lower lethal concentration compared to ethanolic extract. The aqueous concentration is active than ethanolic ones ($p< 0.05$).The positive control with low concentration kills 100% of *Alma emini*. Water considered as negative extract, did not react with helminthes (0% of death).

The determination of LC_{50} and LC_{100} done by probit equation show in theregression equation presented in table 3.

Table 3. Determination of LC_{50} and LC_{100} and regression equation of aqueous and ethanolic extracts of *Myrica salicifolia* and *Psychotria palustris* against *Alma emini*.

Plants	Solvent of extraction	LC_{50}	LC_{90}	Regression equation	R^2 value
Myricasalicifolia	Water	0.83	0.93	Y = 79.148 X -16.027	R^2= 0,93
	Ethanol	2.36	4.29	Y = 20.754 X -1.0014	R^2= 0.98
Psychotriapalustris	Water	5.83	9.40	Y = 11.202 X – 15.313	R^2= 0.98
	Ethanol	21.33	35.79	Y = 2.766 X – 9.016	R^2= 0.99

The LC_{50} and LC_{100} determined by probit equation varied according to plant species and extractive solvent. Water solvent has lower concentration of antihelmintic activity compared to ethanolic solvent in both plants species. *Myrica salicifolia* has high anthelminthic activity than *Psychotria palustris* in the two extractive solvent ($p<0.05$).

As *Myrica salicifolia* has high anthelmintic activity, flavonoids was extracted and the separation of flavoinoids revealed two marks which have different position when using ethyl acetate, methanol and water as elute solvent and developed by aluminium chloride ($AlCl_3$) at 2 % mixed in methanol 95 % (Figure 1).

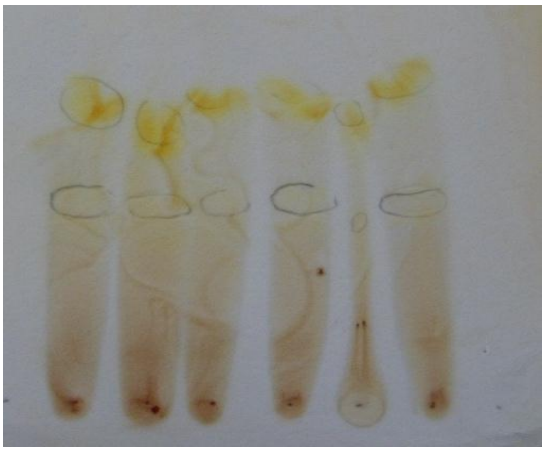

Figure 1: Chromatography of *Myrica salicifolia* developed by ethyl acetate, methanol and water.

TLC was used to ascertain the number of constituents present in the extract and to determine their purity. As well as to determine the solvent mixture that will affect the separation of the components. Subsequently, solvent system composed of *n*-butanol–acetic acid–water at a

volume ratio of 4:1:5 (v/v/v) was tested and results are presented in figure 2.

Figure 2: Chromatography of *Myrica salicifolia* developed by BAW.

Legend: Fl: Flavonoid extract, Aq: Aqueous extract, Et: Ethanolic extract and Met: Methanolic extract

Although the peak resolution was improved, flavonoids were found in all aqueous, methanolic and ethanolic extract in *Myrica salicifolia*.

The determination of anthelmintic activity of different fractions obtained after chromatography column separation is presented in table followed.

Table 4: Mortality rate of flavonoids fractions.

Concentration (mg/mL)	*Myrica salicifolia*	
	Fraction 1	Fraction 2
10	0	100
1	0	100
0.1	0	0

This table shows that the flavonoids fraction 2 is active then the fraction 1 of *Myrica salicifolia*. The concentration of 1 mg/mL of flavonoids for fraction 1 is active to *Alma emini* in vitro. The concentration of 0.1 mg/mL is not active to *Alma emini*.

DISCUSSION

As far as ascertained, this is the first scientific evidence of the anthelmintic activity of *Myrica salicifolia* and *Psychotria palustris* bark, which may be mainly attributed to its tannin content.

Phytochemical analysis of the crude extract has revealed tannins to be among the chemical constituent contained within the two plants. Tannins were shown to produce anthelmintic activities (Niezen et al., 1995; Kahn and Diaz Hernadez, 1999; Athnasiadou et al., 2001; Waller et al., 2001). However, the anthelmintic effect of plants containing tannins actually depends on the type and content of tannins in the plant (Niezen et al., 1998; Athnasiadou et al., 2001).Tannins are polyphenolic compounds (Bate-Smith, 1962) and some synthetic phenolic anthelmintics are niclosamide, oxyclozanide, bithionol, nitroxynil that have been used. Polyphenols which are present in some bryophytes plant have shown to have anthelmintic activity against *Nippostrongylus brasiliensis* (Gamenara et al., 2001). In *Myrica salicifolia* and *Psychotria palustris* it was observed that tannins have strong positive reaction. This activity observed against *Alma emini* can be due to the presence of this secondary metabolite.

Though, condensed tannins have been reported to exert direct anthelmintic effects, other phytochemicals like alkaloids, flavonoids and triterpenes (Anjaneyulu and Prasad, 1982; Gary and Kasera, 1983; Tripathi et al., 1992; Irobi et al., 1994; Brantneret al., 1996; Lahlou, 2002) similar to that found in *Myrica salicifolia* and *Psychotria palustris* may also have their independent or synergistic effect. In the investigated two plants these secondary metabolites have positive reaction or strong positive reaction and may have contributed the anthelmintic activity observed in these plants (e.g. Kamungu et al., 2015b).

The concentration of the active principles in plants may vary in relation to the location, age, and development stage of these plants and whether the plants are freshly harvested or preserved (McCorkle et al., 1996; Habtemariam et al., 1994). This study suggests that ethanolic and aqueous extracts showed comparable anthelmintic activities as standard drug decaris, mebendanzole and albendazole. The lethal concentration of standard drugs except for Decaris were lower than concentration of aqueous and ethanolic extract of aqueous extract of *Myrica salicifolia*, killing 100 % of the earthworms as also reported by Bahizire et al., (2015).

Lethal concentrations (LC_{50} and LC_{100}) of aqueous extract of both plants species are more active than the ethanolic concentrations. This was also observed in the extracts of *Galiniera coffeoides* and *Viscum congolensis* (Bahizirhe et al., 2005). The plant *Myrica salicifolia* contained more anthelmintic virtues than *Psychotria palustris*. But lethal concentrations (LC_{50} and LC_{100}) of *Galiniera coffeoides* and *Viscum congolensis* were more active than that of *Myrica salicifolia* and *Psychotria palustris* (Bahizire et al., 2015).

CONCLUSION

Our investigation of wild chimpanzees diet may serve as a guideline to discovering plants with bioactive properties that should be preserved from destruction because of their health maintenance value for great ape populations. The findings from the current study revealed that extracts from *Myrica salicifolia* and *Psychotria palustris* have shown promising *in vitro* anthelmintic activity against *Alma emini* earthworm. The aqueous extracts are more effective than ethanol extracts. This justifies the mode of administration of these products by the traditional healers and even their use by apes in the forest possibly for self-medication purpose in Kahuzi. The preliminary phytochemical tests revealed the presence of flavonoids, glycosides, steroids, alkaloids, phenolic compounds, saponines, quinones and tannins. The results of the present paper demonstrated that BAW solvent is very useful eluted solvent for flavonoids identification from *Myrica salisifolia.* More detailed analyses should deal with extraction of different active constituents contained in the plants in order to assess their therapeutic role in different species of parasites infecting wild animals in the park.

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Authors' contributions

KS and BM designed and performed the experiment. BM and BK analyzed the data. BK and BM had given technical guidance during the experiment, drafted and revised the manuscript. All authors read and approved the final manuscript.

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