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Ethnobotanical survey and phytochemical screening of aqueous extracts from *Detarium microcarpum* Guill. & Perr in Mali

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Detarium microcarpum **Guill. & Perr is a tree which belongs to Caesalpiniacea family and can reach up 10 m of height, with a horizontal root system. It is confined to the arid regions of West and Central Africa, where it mainly occurs in savannah. The aim of this work is to collate information on the use of *Detarium microcarpum* in folk medicine by traditional health practitioners and herbalists. Moreover, a phytochemical screening was carried out on the aqueous extracts from its major organs. The ethnobotanical survey was conducted in three administrative regions of Mali (Koulikoro, Sikasso, and Segou), and in the District of Bamako involving 45 individuals, including 25 women and 20 men. According to the collected information leaf, bark, and root of the plant have therapeutic properties and are known to treat 16 diseases, mainly infections and pains. The phytochemical screening detected numerous secondary metabolites, including alkaloids, polyphenolic compounds, terpenes, quinones, anthraquinones, reducing sugars, amines and saponosides in aqueous extracts from leaf, stem bark and root. These compounds could justify the use of this plant in folk medicine.**

Key words: *Detarium microcarpum,* ethnobotanical survey, phytochemical screening, aqueous extracts, Mali.

INTRODUCTION

The relation between man and his quest for plant drugs dates back to ancient times, as witnessed by numerous written documents, preserved monuments, and even original recipes of plant medicines (Petrovska, 2012). Observations in free-living chimpanzees in Nigeria that use specific plants for healing purposes indicate that plant medicinepre-dating humanity (Fowler et al., 2007).

The wealth and diversity of medicinal plants has remained important since, an estimated 80% of the world population does not have access to modern medicine

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and has to rely entirely on the local traditional medicinal plants. This reservoir has remained largely untapped. The number of known plant species is estimated to be around 380,000(Christenhuz and Bing, 2016) but only 10% of them have been the subject of more or less extensive scientific investigations (Badiaga, 2011). One strategy to find new plants of medicinal value and to identify new bio-active compounds is to investigate traditional systems of medicine. While Traditional Chinese Medicine, Ayurvedic Medicine, and Arabic Medicine have attracted considerable attention, the traditional healing systems of Africa have been systematically underrepresented. This is astonishing, since several regions of Africa are considered as biodiversity hotspots, especially sub-Sahara regions of Western Africa which belong to the regions of the world with the highest density of plant species (Küper et al., 2004).

Within the diverse and rich African flora, certain plant taxa are of particular interest for their medicinal properties and their important role in traditional healing practices in tropical Africa. The genus *Detarium* belongs to these taxa of high potential. Based on the size of the leaflets, the density and the shape of the secretory pockets visible as translucent spots in the mesophyll of the leaf blade, and the features of the inflorescence, three species can be distinguished: *Detarium microcarpum* Guill & Perr, a species of the savanna, *Detarium senegalense* J.F. Gmelin, a forest species, and Detarium macrocarpum Harms, inhabiting the humid forests of tropical Africa (Akah et al., 2012). While all three Detarium species are used for folk medicine, D. microcarpum dominates under the very arid conditions of Mali. This tree, reaching 10 m of height and endowed with an horizontal root system, is confined to the dry regions of West and Central Africa, occurring in tropical forests and drier savannahs (Leung et al., 1968; Abubakar et al., 2017). In Mali, D. microcarpum is well known as "N'tabacoumba" and intensively used in the traditional pharmacopoeia for its multiple therapeutic properties, (Kouyaté and Lamien, 2011), similar to most West African countries the fruits, leaves and flowers of *D. microcarpum* are consumed as food, while its leaves, bark, roots and almonds, are used in traditional medicine (Kouyaté, 2005, Dieng et al., 2019).

One of the main medicinal uses of D. microcarpum is the treatment of diarrhoea, meningitis, tuberculosis and haemorrhoids (Hassanin et al., 2019; Kurmi et al., 2021) but its applications are not confined to this. For example, the infusion of bark of D. microcarpum has diuretic, anti-inflammatory and anti-parasitic properties, and fruits and leaves of this species are used in the treatment of dysentery (Iwu, 1993; Ikhiri and Ilagouma, 1995). A decoction of the roots is used as a drink against syphilis, while the macerated roots are used for the treatment of stomach aches. The decoction of D. microcarpum leaves is used to treat stomach aches, toothaches, and difficult delivery (Kouyaté, 2005) and the decoction of the stem bark are drunk to cure simple or bloody diarrhea. Moreover, some studies have revealed its antiblennorhagic (Kouyaté, 2005) and antimicrobial activities (Ouedrago, 1997; Kouyaté et al., 2002). In Nigeria, the bark, leaves and roots are prepared as medication to treat skin infections, diarrhea and menstrual pain, rheumatism, urogenital infections and haemorrhoids (Dahiru et al., 2022).

In addition to the manifold medicinal applications, *D. microcarpum* serves as thickener of traditional soups. Since it contains lipids, carbohydrates, proteins, crude fibers and essential elements such as Na, K, Mg, K, S, P and Fe (Abreu and Relva, 2002) as well as substantial amounts of vitamin C and dietary sugar (Kouyaté et al., 2008). This plant can also be considered as a traditional form of functional food.

While ethnobotanical use and potential chemical composition of *D. microcarpum* have been investigated, regional differences have, to the best of our knowledge, not been systematically addressed. The wide range of different and quite diverse medicinal activities leads to the question, if geographic differences, either in use of certain plant parts, the method of extraction, and possibly chemical composition might play a role. Therefore, the aim of this work is to conduct a comparative ethnobotanical survey on the medicinal uses of *D. microcarpum* in different populations in different regions of Mali, and then to conduct a phytochemical study of the organs harvested, in order to correlate characterised constituents and traditional practices.

The novelty of this approach is to link differences in plant use with potential differences in compound profile or abundance as contribution to valorise *D. microcarpum* and to contribute to quality assessment of traditional medicine.

MATERIALS AND METHODS

Leaves, bark, and total roots of *D. microcarpum* were collected from Tabarako, Sikasso and Bougoula , between October to November 2018. The material was authenticated by the Department of Traditional Medicine (DMT) of the National Institute of Public Health (INSP) in Bamako (Mali) under the registration number 3053/DMT.

The region of Sikasso is located in the extreme south of Mali with 2,977,595 inhabitants. The region consists of hills and mountains in the south, valleys and plains in the center and north. The climate is of the Sudanian tropical type. It is the wet region of Mali. Vegetation cover ranges from wooded savannah to gallery forest. The economy of Sikasso is mainly based on agricultural production (cereals and fruits, cotton cultivation and tea culture) and mining production. The sub-soil is rich in minerals. The region is the second industrial region of Mali, after Bamako. Geographical coordinates of Sikasso is 11° 11′ 59″ north and 7° 05′ 49″ west.

Sanankoroba is a commune in the region of Koulikoro located in the south of Bamako with a tropical climate. Its territory is made up of vast plains bordering the Niger with some lateritic plateaus to the east and south. This commune has a multi-ethnic population estimated at 37,294 inhabitants. Agriculture is its main economic activity supported by animal husbandry, fishing, gathering and market gardening (Djiré, 2004). Geographical coordinates of Sanankoroba is 12° 23′ 51″ north and 7° 56′ 22″ west.

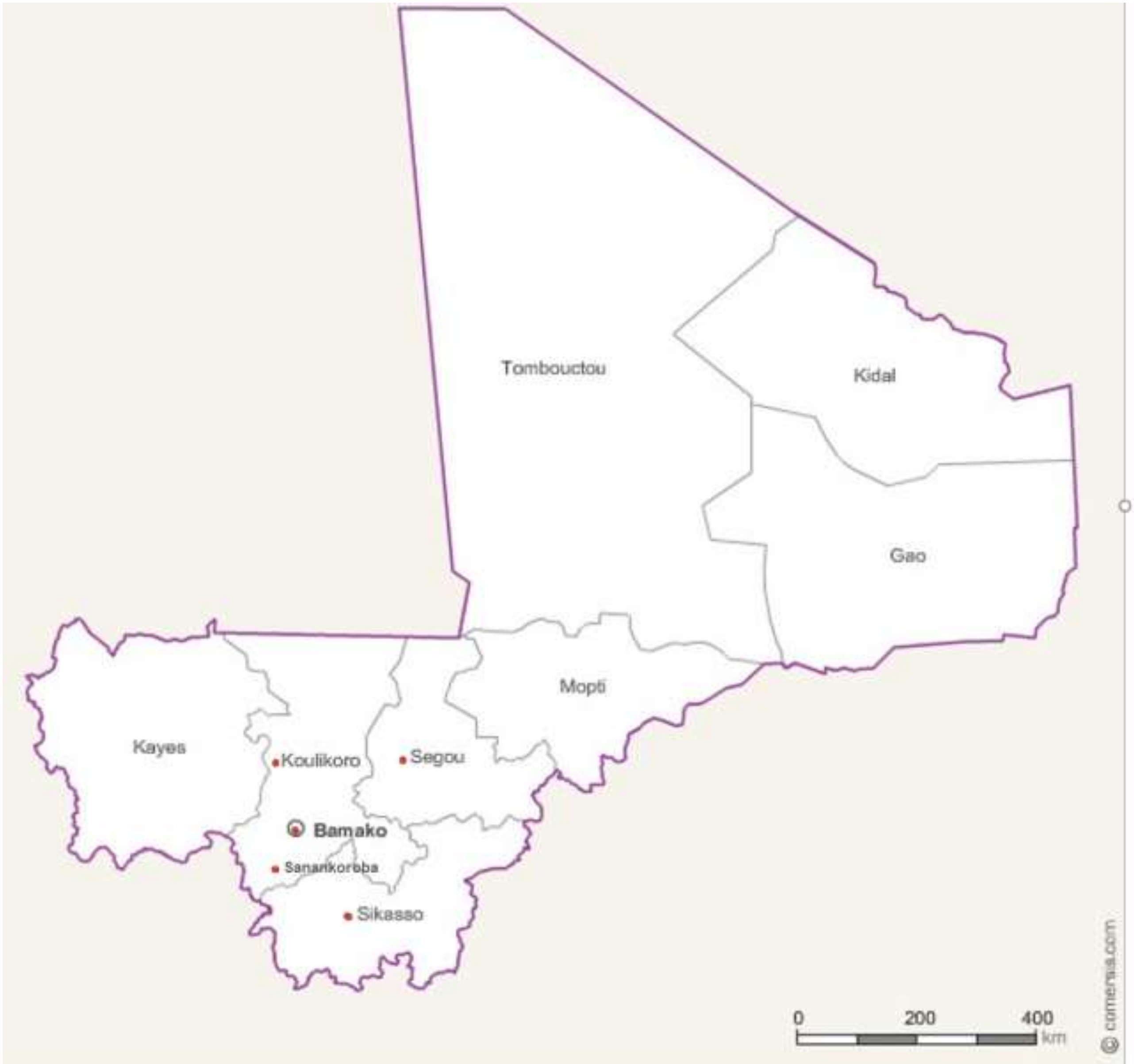

Figure 1. Map of Mali with harvesting sites.
(Authors, 2022; Comersis, 2022)

METHODS

Selection of locations for survey and harvest

The localities of the ethnobotanical survey (Figure 1) were selected due to their well-developed tradition in herbal medicine and the harvest localities were chosen for their contrast with respect to climatic conditions, while providing abundant vegetation, especially for the occurrence of *D. microcarpum* and a low level of pollution.

Ethnobotanical survey

The study method is based on an ethnobotanical questionnaire already established in a previous study (Somboro et al., 2011), which was handed over to the respondents during the individual interviews and contained thirty eight questions. The first part of the questions relates to the personal profile of each respondent (age, gender, education, residence and habitat); the second part of the questionnaire ask for the actual ethnomedical activity (such as the local name of the species, and the different organs used, as well as the methods of preparation and administration). The survey was conducted in the period between September to December, 2018.

Phytochemical screening

Phytochemical screening was performed on aqueous extracts in order to highlight the presence or absence of certain classes of secondary metabolites using colorimetric assays.

Table 1. Uses in traditional medicine of different parts of *D. microcarpum* Guill. & Perr.

Diseases treated	Mode of use	Plant part used	Site
Difficult delivery	Decoction	Leaves	Bamako
Muscle pain	Decoction	Root and leaves	Segou
Dysentery	Infusion	Leaves	Bamako
Open fontanels	Decoction	Leaves	Bamako
Unknown diseases	Decoction	Leaves	Segou, Bamako, Sikasso
Snake bite	Decoction	Leaves	Bamako
Cardiac disease	Decoction	Leaves and Bark	Sanankoroba
Painful periods	Decoction	leaves and bark	Segou
Witchcraft	Decoction	Leaves	Segou, Bamako, Sikasso, Sanankoroba
Bilharzia	Decoction	Bark	Bamako
Infections	Decoction	Bark, roots and fruits	Bamako
Syphilis	Decoction	Bark, roots and fruits	Bamako, Segou
Bellyaches in women	Maceration	Root	Segou
Onchocerciasis	Decoction	Root	Segou
Fever	Consume regulary	Fruit	Sanankoroba
Meningitis	Consume once a year.	Fruit	Bamako

Source: Authors 2022

Preparation of aqueous extracts

20 g of powered plant material was placed in 200 mL of distilled water and boiled under reflux while stirring for 15 min (Kasmi et al., 2017). The obtained solution was filtered under vacuum after cooling. The filtrate obtained was evaporated to dryness in the oven for 24 h, the solid residue was ground and stored at 4°C awaiting further analysis for the phytochemical assays described. Prior to analysis, the powder was dissolved in hot water to a concentration of 10 mg/mL. This solution is designated in the following as "extract".

Detection of alkaloids

The presence or absence of alkaloids was confirmed by two tests. To 2 mL of the aqueous extract, 5 mL of 1% HCl were added, and incubation at 35°C in a water bath for 15 min, before dividing the extract into two equal parts. The first part was supplemented with 5 drops of Mayer's reagent (1.36% w/v mercuric chloride and 5% w/v in water) leading to a white precipitate in presence of alkaloids. The second part was supplemented with 5 drops of Wagner's reagent (2% w/v of iodine and 6% w/v of KI in water), yielding a brown precipitate, if alkaloids are present. The presence of alkaloids is confirmed by the formation of white or brown precipitate (Mojab et al., 2003).

Flavonoids

One milliliter of the aqueous extract was treated with a few drops of concentrated HCl, adding a few milligrams of magnesium chips. The presence of flavonoids was reported by the appearance of a red, orange or pink color (N'Guessan et al., 2009).

Tannins

One milliliter of the aqueous extract was complemented with 2 to 3 drops of 1% w/v $FeCl_3$. After a few minutes of incubations at room temperature, tannins were reported by the appearance of a strong blue or green (Karumi et al., 2004).

Sterols and triterpenes

To one milliliter of the aqueous extract, 1 mL of acetic anhydride and a few drops of concentrated H_2SO_4 were added. The appearance of a purplish to green or brownish coloration of the interface layer indicates the presence of triterpene saponosides (Edeoga et al., 2005).

Quinones

To one milliliter of the aqueous extract, a few drops of 1% NaOH are added. Free quinones will cause the appearance of a colour that turns yellow, red or purple (Kasouni et al., 2021).

Terpenoids

One milliliter of the aqueous extract are complemented with 0.4 ml $CHCl_3$ and 0.6 ml of concentrated H_2SO_4, which will in the presence of terpenoids produce two phases and a brown colour at the interphase (Khan et al., 2011).

Anthraquinones

To one milliliter aqueous of extract, 0.5 mL of 10% NH_4OH are added, and the mixture is shaken. The appearance of a purple colour indicates the presence of anthraquinones (Oloyede, 2005).

Reducing sugars

To five milliliter of extract, 1 mL of Fehling's liquor (Fehling, 1849) is added. Then the mixture is heated in a water bath at 70°C for 5 min. The appearance of a brick- red coloured precipitate reports the presence of reducing sugars (Cai et al., 2011).

Amines

A drop of extract on a filter paper is dried at 80°C in the oven, and then complemented by a drop of ninhydrin (0.5% w/v of ninhydrin in 65% ethanol), drying again in the oven at 110°C for 5 min. The appearance of a purple spot indicates the presence of amines (Kasouni et al., 2021).

Saponosides

To one milliliter of extract, 2 mL of hot distilled water are added, and the mixtures is then shaken for a few seconds and then left to stand for 15 min. A foam height of more than 1 cm persisting over this time indicates the presence of saponosides (N'Guessan et al., 2009).

RESULTS AND DISCUSSION

Ethnobotanical survey

To collect the ethnobotanical data, 45 people, including 25 women and 20 men with different social characteristics between the ages of 30 and 70 years, were interviewed orally using a questionnaire addressing 38 points orally. The data collected are divided into the following tables.

Bark decoction, taken orally and used as body bath, is cited as a general treatment for infections in Bamako. In Sanankoroba, a mixture of bark and root decoction is used for this purpose. Syphilis is treated with a root decoction in Segou, while in Bamako, the fruit is added (Table1). All respondents in Bamako, Segou and Sikasso unanimously emphasized that the decoction of *D. microcarpum* leaves used as a drink and bath is effective against unknown diseases (witchcraft, black magic).

Most applications (25% in total) involve leaves of *D. microcarpum*. The preferential use of the leaves compared to the other parts of the plant in the treatment of diseases has also been highlighted in previous studies (Koné, 2009). This could be explained that leaves are easy to access and regenerate. Mixtures of leaves and bark accounts for 17% of applications, as well as the bark from the stem. Instead root, fruits, combinations of roots and trunk bark, combinations of leaves and root, or combinations of roots and fruits are used more rarely (8% of cases). The overwhelming majority (90%) of applications uses decoction as mode of preparation, only 10% is based on mere maceration, infusions do not seem to play a role. One of the reasons for the predominant use of decoctions is the hard consistency, especially of roots, or bark, to some extent also of leaves. In addition, decoctions support to increases the temperature in the organism, supporting healing (Lahsissene et al., 2009), and allow for a certain fractionation of the water soluble active compounds from less soluble and partially toxic compounds, thus attenuating noxious side effects of certain plant drugs (Salhi et al., 2010). Overall, the oral route along with body bath is the main modes of application, while local application through the skin and the sitz bath are more rarely used.

Among the 16 diseases that are cured by D. microcarpum, pains and infections are the most cited. The results of our survey are congruent with previous studies. For instance, the preferential administration through the oral route was also reported by Assouma et al. (2018), Kpabi et al. (2020) and Togola et al. (2020). The use of D. microcarpum against syphilis and an incense from seed against witchcraft was also noted by Akah et al. (2012), as well as leaf decoctions remedy for childbirth complications. Consumption of the raw fruit as protection against meningitis was already quoted by Loubaki et al. (1999), and the decoction of the powdered bark to mitigate painful menstruation was described by Kouyaté (2005). Despite the difference in study sites, there is a good degree of consistency in the information collected from herbalists and traditional health practitioners. While many aspects of the current survey agree with findings from previous studies, some aspects turned out to be new. These included the use of *D. microcarpum* to treat cardiovascular diseases, onchocerciasis and fontanel opening in infants.

Phytochemical screening

The phytochemical analysis detected alkaloids, quinones, tannins, flavonoids, sterols, triterpenes, anthraquinones, reducing sugar, terpenoids and amines in all aqueous extracts, independently of geographic origin and independent of the source tissue of *D. microcarpum*. The absence of saponosides from all tested extracts was noted (Table 2).

Previous studies had detected tannins in aqueous extracts (Loubaki et al., 1999; Sereme et al., 2008), flavonoids and alkaloids in methanolic extracts of D. microcarpum bark (Friday et al., 2018; Okolo et al., 2012), and saponins, reducting sugars, flavonoids, terpenoids and steroids in methanolic extracts of the roots (Khan et al., 2011). Except for saponosides, our results are in concordance with those data. This difference might be linked with the extraction solvent could also have an influence on the presence of secondary metabolites, since the comparison of *n*-hexane versus methanolic extracts from stem bark showed that the first solvent could recover only few of the compounds found in the methanolic extract (Zakari and Kubmarawa, 2016). Likewise, hydroethanolic extracts of D. microcarpum roots, root bark, leaf and twig extracts revealed the presence of phenolic compounds, flavonoids, sterols, triterpenes, glucosides, coumarins, and saponins, while alkaloids and tannins were absent (Mbock et al., 2020). Also for methanolic leaf extracts of

Table 2. Phytochemical screening of the aqueous extract of different organs of *D. microcarpum*.

Chemical group	Sikasso			Sanankoroba		
	Leaf	Bark	Root	Leaf	Bark	Root
Alkaloids	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Sterol/triterpenes	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Quinones	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+
Anthraquinones	+	+	+	+	+	+
Reducing sugars	+	+	+	+	+	+
Saponosides	-	-	-	-	-	-

+ : presence, -: absence.
Source: Authors 2022

D. microcarpum, terpenoids, flavonoids, saponins, tannins and steroids were detected (David et al., 2017), in a different study carbohydrates, flavonoids, alkaloids, tannins, saponins, steroids and triterpenoids (Kurmi et al., 2021).

Whatever the route of administration (oral or local), the antibacterial and antifungal effects of tannins are well established (Bruneton and Poupon, 2016), which might be the molecular base for the use of D. microcarpum against the various bacterial infections mentioned above. Since some tannins (especially gallic tannins) have an anti-diarrheal activity, these compounds might also account for the prescription of D. microcarpum to treat severe diarrhea and dysentery.

Many flavonoids, due to their antioxidant properties can mitigate inflammations (Bruneton and Poupon, 2016). The occurrence of flavonoids in the different parts of D. microcarpum might provide a mechanistic base for the effect of this tree against pain, tiredness, as well as venolymphatic perturbations.

Many polyphenols are known to be cardioprotective (Ganesan and Xu, 2017) and might be the base for the respective activity of D. microcarpum.

Conclusion

This work connected the traditional use of *D. microcarpum* for the treatment of different diseases with phytochemical profiling. Information on sixteen ailments was collected where all major organs of this plant were employed, mostly using decoction as preferred method of preparation. The phytochemical screening detected the presence of numerous secondary metabolites, including alkaloids, polyphenols, sterols, and tri-terpenes, quinones, and reducing sugars, which can explain the therapeutic power of this plant. In contrast to previous studies, the study was not able to detect any saponins. Although this plant is extensively used in traditional medicine, so far, differences with respect to geographical location correlating ethno-pharmacological use with phytochemical composition of the different organs have not been addressed scientifically to the best of our knowledge results to composition of the various organs of this plant harvested on various sites. While the two study sites differed considerably with respect to climatic conditions, the ethno-pharmacological use displayed a high degree of congruence. This was accompanied by a very strong qualitative overlap with respect to the phytochemical profiles from the two sites. If both, human use for healing and phytochemistry remain consistent against a considerable difference of geography and environment, this lends strong support for common phytochemical principles behind the medicinal use. In the current studies, the molecular base for the bioactivities of D. microcarpum was therefore explored.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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