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Full Length Research Paper

An ethnobotanical and phytochemical study of the African medicinal plant *Guiera senegalensis* J. F. Gmel

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The Sudano-Sahelian species *Guiera senegalensis* J. F. Gmel. is a small shrub found mainly in West Africa. It is well known in the Sahel, where it grows gregariously, forming abundant single-species colonies on fallow clay or sandy soils. Its widespread use in traditional medicine prompted us to conduct an ethnobotanical survey and phytochemical screening of this plant. The chemical analysis tests we carried out confirmed the results of earlier work. The main classes of secondary metabolites, namely anthraquinones, terpenoids, saponins, alkaloids, coumarins, mucilages, flavonoids, tannins, and cardiotonic and cyanogenic heterosides were assayed in different organs of the plant: leaves, fruits, and root and stem bark. New uses of different parts of *G. senegalensis* by the traditional medical practitioners were recorded especially the use of decoctions of leaves to relieve abdominal pain and migraines. The further investigation of the potentially active constituents of *G. senegalensis* should provide new potential medicines for the phytotherapeutic arsenal.

Key words: *Guiera senegalensis,* ethnobotanical study, chemical characterisation, assay.

INTRODUCTION

Guiera senegalensis, very well known in its native area, generally occurs as a shrub that can grow to a height of 3 to 5 m according to habitat. Its stem presents numerous knots that send out branches. The ash-grey stem and branches have fibrous or pubescent bark and bear opposing, short petiolated oval leaves, sometimes mucronate, sometimes even cordate at their base, about 2 to 4 cm long by 1 to 2 cm wide. These grey-green leaves, darker on their upper surface, display black spots on their lower surface and are slightly downy on both sides. These features lend the plant an overall silver-green colour that is conspicuous in brushland (Silva et al., 2008). The literature reports several recorded uses for *G. senegalensis* in traditional medicine to treat various illnesses (Fiot et al., 2004). It is recognised as being active against cough, respiratory congestion and fever (Kerharo and Adam, 1974), and is prescribed as an antitussive (Negrevergne, 1968; Faye et al., 1980; Sanogo et al., 1998b; Diatta et al., 2007), to ease breathing and to treat lung and bronchial disorders. It is also used against malaria (Benoit et al., 1996; Ancolio et al., 2002; Azas et al., 2002). It is often prescribed in combination with *Combretum micranthum* or *Combretum*

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glutinosum (Kerharo and Adam, 1974).

The branches, leaves, bark and roots of *G. senegalensis* are also recommended for the treatment of stomach pain and dysenteric diarrhoea (Kerharo et al., 1948; Aniagu et al., 2005), syphilis, beriberi, leprosy and impotence (Kerharo et al., 1948). It is also used in veterinary medicine among the Tukolor people in diets designed to increase body weight, reproductive capacity and milk secretion in animals (Kerharo and Adam, 1974).

It is used externally as an antiseptic healing preparation for wounds, stomatitis, gingivitis and syphilitic cankers (Kerharo and Adam, 1974). Some populations mix galls from *G. senegalensis* (gall nuts are frequently formed on the above-ground parts of the plant) with charcoal to make a highly diuretic powder prescribed in serious cases of oligouria and even anuria, and in particular for cerebral malaria. It has also been demonstrated that preparations made from galls possess antiviral properties (Lamien et al., 2005b, 2005c; Aniagu et al., 2005).

Other people reduce the galls to powder with the pith from *Combretum aculeatum* stems and salt. This powder is diluted in water immediately before use and is prescribed for painful stomach cramps with mucous stools and vomiting (Kerharo and Adam, 1974).

A tea made from leaves is prescribed by the oral route to treat eczema (one litre per day), and is also used against attacks of fever, and to cure chest conditions and colds (Malgras, 1992). Fresh mashed or chewed or cut leaves when placed on a wound staunch bleeding (Berhaut, 1967).

Powdered dried leaves associated with *Melanthera scandens* are administered by the nasal route to treat headaches and sinusitis (Berhaut, 1967). The leaves are also used as a poultice on tumours and against the Guinea worm (Berhaut, 1967). Besides these uses in traditional medicine, some extracts of this plant have been found to possess pharmacological properties: antimicrobial (Le Grand, 1989; Bosisio et al., 1997; Sanogo et al., 1998a), antifungal (Silva and Elsa, 2003), antioxidant (Bucar et al., 1998; Bouchet, 1998) and trypanocidal (Aderbauer et al., 2008). Effects on the central nervous system (Amos et al., 2001), on cancer cells (Bucar et al., 1996) and as a snake venom detoxicant (Abubacar, 2000) have also been described.

Although the wide range of uses in traditional medicine is often mentioned in the literature, few ethnobotanical surveys (Fiot et al., 2004) or complete phytochemical studies (Fiot et al., 2006) have been published to date. We set out to conduct an ethnobotanical survey among the populations at three targeted locations in Mali, and to make a comparative phytochemical study of the different plant organs (leaves, fruits, and root and stem bark) collected at each location and used for traditional medicinal preparations.

A correlation between the components characterised in the different plant organs and their traditional use could then be sought.

MATERIALS AND METHODS

Country and plant material

Mali is a West African country located between latitudes 10 and 25° north and longitudes 4 to 12° west. Its area is 1,241,238 km^2. Mali is landlocked and has frontiers with seven countries: Algeria to the north, Ivory Coast and Guinea to the south, Niger and Burkina-Faso to the east and Mauritania and Senegal to the west, in all 7000 km of frontiers with neighbouring states.

The vegetation of Mali is of the Sudano-Sahelian type. The country is mostly flat (highest point 1200 m) and its climate is subtropical to arid. It is swept by the harmattan, a hot, dry wind, and the moist monsoon wind, which brings rain. For this work we selected three survey and harvesting locations along a rainfall gradient: Badougou Nafadji in the region of Koulikoro, in the south-east of the country, has a Sudano-Guinean climate with a pluviometric index of 950 to 1250 mm and a single rainy season lasting 5 months (mid-May to mid-October). The local vegetation type is savanna.

Daral, also in the Koulikoro region, but north-east of Badougou Nafadji, has a Guinean climate with a pluviometric index of 750 to 950 mm. The single rainy season lasts about 4 months (June to September) and the local vegetation type is tropical savanna.

Ségué, in the Mopti region, has a moderately arid climate of the Sahelian type, with a pluviometric index of 500 to 750 mm. The rainy season lasts at most 3 months (July to September) and the vegetation type is thorn- and grass-steppe.

This climate range enabled us to study the effect of climate on the chemical composition of the different plant organs. The different organs of *G. senegalensis* (leaves, fruits, root bark and stem bark) were harvested between 31 January 2006 and 1 March 2006 at each of the three chosen locations.

These plant samples were identified by the Department of Traditional Medicine, Bamako, Mali. The specimens are available in the Department's collection under the numbers 0536, 0537, 0749, 2212 and 2254. The organs were dried for two weeks at ambient temperature and ground in a mortar.

Ethnobotanical surveys

Surveys were conducted between January and March 2006 among the populations and traditional medical practitioners at the three selected harvesting locations. In all, 31 traditional medical practitioners were questioned; 10 at Badougou Nafadji, 5 at Daral and 16 at Ségué. This variability according to location reflected how close the location was to an urban centre and also the number of inhabitants. Badougou Nafadji is 40 km from Bamako, the capital, and has a population of 1700. Daral is 15 km from Bamako-Kati, with a population of 150 to 200, and Ségué is 700 km from Bamako and 125 km from its regional capital Mopti, with a population of more than 2000.

An interview guide with eight questions was used to collect information from the traditional medical practitioners concerning knowledge of the plant and set modes of preparation.

(1) What is the local name for *G. senegalensis*?
(2) Is this plant used medicinally?
(3) What diseases are treated with this plant?
(4) Which part of this plant (leaves, fruits, flowers, stem bark, root bark, gall, mistletoe, total roots) is used in the treatment?
(5) Is this remedy used fresh or dried? If used dried, how is it dried? In the sun or in the shade?
(6) How is the remedy prepared?
(7) How much plant material must be used or prepared, in how much water, and for how long (decoction, maceration, hot steeping, digestion, etc.)?

(8) Is *G. senegalensis* used alone for medicinal purposes? Or is it combined with other plants?

Chemical analysis

Tests for chemical analysis were carried out on powders prepared beforehand from each plant organ using conventional test reagents (Paris, OUA CSTR, 1985; Bruneton, 1993).

Water content

For this study we referred to the method used at the Department of Traditional Medicine, Mali. Too high a water content (>10%) in the plant material favours the enzymatic hydrolysis of certain constituents, which can degrade them. It was therefore important to determine the water content of each sample. Two methods were used: azeotropic and gravimetric.

The azeotropic or volumetric method involves removing the water contained in the powder by distillation with toluene, which forms an azeotrope with water. In a flask fitted with a Dean-Stark receiver, 100 ml of toluene and 1 ml of water were refluxed for 1 h. The volume of water collected was V_1.

A 5 g sample of plant powder was then added to the flask and the mixture refluxed for 1 h. The total volume of water collected was V_2. The percentage water content P_w is given by:

$$P_w = (V_2 - V_1) \times 100/5$$

The gravimetric or weight method uses desiccation to measure the amount of water contained in the material. An exact quantity M_1 of plant matter was weighed on a watch glass, which was then heated at 105°C in a desiccator for 24 h. The plant matter on the watch glass was weighed again, giving M_2. The percentage water content P_w is then given by:

$$P_w = (M_2/M_1) \times 100.$$

Ash assay

The quantity of non-volatile residual substances in the material was measured by ashing. The total ash assay was performed by calcining a quantity M_1 (about 2 g) of plant matter in an oven at 800°C for 6 h. A mass M_2 of ash was obtained. Total ash as a percentage C_T is given by:

$$C_T = (M_2/M_1) \times 100.$$

The amount of silicaceous matter in the plant material was evaluated by measuring the ash that was insoluble in hydrochloric acid.

To the total ash obtained previously was added 20 ml of 10% hydrochloric acid. The mixture was heated for 15 min on a water bath and filtered on ashless paper. The filtrate and the paper were then incinerated. A mass M_3 of ash insoluble in hydrochloric acid was obtained. The amount of silicaceous matter as a percentage C_{Cl} is given by:

$$C_{Cl} = (M_3/M_1) \times 100.$$

Sulphuric ash was determined by gravimetric assay of non-volatile sulphates obtained by calcining a quantity M_1 of plant matter impregnated with 50% aqueous sulphuric acid. The sample was oven-dried for 24 h and then calcined for 6 h at 800°C. A mass M_2 of sulphuric ash was obtained. Sulphuric ash as a percentage C_s is then given by:

$$C = (M_2/M_1) \times 100$$

Assay of extractible substances

Assay of water-extractible and ethanol-extractible substances gives the percentages of water-soluble and ethanol-soluble compounds present in the plant matter. The water-extractible content was determined by decocting 1 g of plant matter in 20 ml of distilled water for 15 min. The suspension was filtered and the filtrate was evaporated to dryness and weighed (M). The percentage of water-extractible substances P_s is given by:

$$P_s = M \times 100$$

The ethanol-extractible content was determined analogously, using 20 ml of 96% ethanol. The percentage of ethanol-extractible substances is noted P_E.

Alkaloids

The presence of alkaloids was shown by precipitation of salts and reaction with Mayer's reagent (potassium tetra-iodo mercurate solution). To 10 g of powdered sample, 50 ml of 10% sulphuric acid was added. After 24 h maceration at ambient temperature the mixture was filtered on filter paper and rinsed with distilled water to obtain 50 ml of filtrate, 1 ml of which was placed in a test tube and treated with five drops of Mayer's reagent. A white-yellow precipitate indicated the presence of alkaloids.

Tannins

The presence of gallic and catechin tannins was demonstrated using ferric perchloride (Bruneton, 1993). First 5 g of powdered sample was placed in 100 ml of water and the mixture boiled. After 15 min the suspension was filtered and rinsed to give 100 ml of solution. This solution was also used to characterise flavonoids.

Hydrolysable gallic tannins were detected by adding 15 ml of Stiasny reagent (10 ml of 40% formol + 5 ml of concentrated hydrochloric acid) to 30 ml of the solution. After 15 min heating on a water bath at 90°C the mixture was filtered and 5 g of sodium acetate was added, followed by 1 ml of 1% $FeCl_3$. A blue-black coloration indicated the presence of gallic tannins.

Non-hydrolysable catechin tannins were detected by adding 1 ml of concentrated hydrochloric acid to 5 ml of the previously prepared solution. The mixture was boiled for 15 min. A red precipitate insoluble in amyl alcohol indicated the presence of catechin tannins.

Flavonoids

Flavonoids, which are practically ubiquitous in plants, form a large family of pigments that very often occur in large quantities. Several tests can be used to detect different types of flavonoids (Bruneton, 1993).

The cyanidin reaction reveals the presence of free flavonoids (flavones and dihydroflavonols). To 5 ml of solution prepared using the same procedure as for tannins was added 5 ml of a solution of 50% concentrated hydrochloric acid in ethanol. After addition of 1 ml of isoamyl alcohol and a few magnesium turnings, a pink-orange or pink-violet coloration indicated the presence of free flavonoids. Leucoanthocyans are also detected by the cyanidin reaction carried out without the addition of magnesium shavings. After 15 min heating on a water bath, a red coloration indicated their presence.
Anthocyans were detected by adding 5 ml of 10% sulphuric acid

and then 5 ml of 50% ammonia to 5 ml of solution. If the coloration of the solution deepened on acidification and then turned blue in basic medium, then anthocyans were present.

Anthracenes

Anthracene derivatives are found in plants in the form of genins (or aglycones), free quinones, or as anthracene heteroside combinations. The presence of anthracene derivatives is shown using 50% ammonia (Bruneton, 1993). A chloroform extract was first made from 1 g of powdered sample, followed by heating for 3 min on a water bath. The solution was filtered hot. The extraction residue was used to detect heteroside forms (*O*-heterosides and *C*-heterosides).

Free anthracenes were detected by adding 1 ml of 50% ammonia to 1 ml of chloroform extract, followed by stirring. A reddish coloration indicated their presence.

O-Heterosides were detected by adding to the previous chloroform extraction residue 10 ml of distilled water and 1 ml of concentrated HCl. The mixture was heated for 15 min on a water bath, and the tube was then cooled under cold running water and the contents filtered. To 5 ml of the filtrate was added 5 ml of chloroform, and after shaking, the organic phase was separated. To the organic phase was added 1 ml of 50% ammonia. A red coloration of variable intensity indicated their presence.

C-Heterosides were detected by adding 10 ml of distilled water and 1 ml of 10% $FeCl_3$ to the aqueous phase obtained previously for *O*-heterosides. The mixture was heated for 30 min on a water bath and then cooled under water. The organic phase was separated after shaking with 5 ml of chloroform and 1 ml of 50% ammonia was added. A red coloration of variable intensity indicated their presence.

Sterols and triterpenes

The presence of sterols and triterpenes was demonstrated using concentrated sulphuric acid (Bruneton, 1993). An extract was first prepared by maceration for 24 h of 1 g of powdered sample and 20 ml of ether. The extract obtained was used to detect carotenoids and coumarins in addition to sterols.

Sterols and triterpenes were detected by adding 1 ml of chloroform to the residue left by evaporating 10 ml of the macerate. The solution obtained was placed in two test tubes, and 1 to 2 ml of concentrated sulphuric acid was placed in the bottom of one of them, the other acting as a control. The formation of a brownish-red or violet ring where the two phases met indicated their presence. Carotenoids, terpene pigments useful for photosynthesis, were detected using antimony trichloride (Bruneton, 1993). Two to three drops of a saturated solution of antimony trichloride ($SbCl_3$) in chloroform were added to take up the residue left by evaporating 5 ml of ether extract to dryness. A blue coloration indicated their presence.

Cardiotonic heterosides

The presence of cardiotonic heterosides was demonstrated using potassium hydroxide in the presence of appropriate reagents (Bruneton, 1993). A chloroform phase was first made up from 1 g of powdered sample. To this, 10 ml of 60% ethanol and 5 ml of 10% lead acetate solution were added. After heating the mixture on boiling water bath for 10 min, 10 ml of chloroform was added, followed by shaking. The organic phase was separated and placed in three test tubes. The solution in each tube was evaporated to dryness on a boiling water bath. The residue of each tube was taken up in 0.4 ml of isopropanol. To the first tube was added 1 ml of Baljet reagent, to the second 1 ml of Kedde reagent and to the third 1 ml of Raymond-Marthoud reagent.

To each tube was then added five drops of 5% KOH in ethanol. The appearance after 10 min of an orange coloration in the first tube, a red-violet coloration in the second tube, and a transient violet coloration in the third tube indicated their presence.

Saponosides

Saponosides, which occur widely in plants, are characterised by their foaming power in aqueous solution (Bruneton, 1993). A decoction was first made from 1 g of powder and 100 ml of distilled water. After boiling for 15 min the suspension was filtered. To 10 test tubes were successively added 1, 2, 3, up to 10 ml of the filtrate, topped up to 10 ml with distilled water. Each tube was shaken for 15 s. The height of the foam was measured 15 min after shaking. The foaming index I_m was calculated from the tube number (N) in which the foam height was 1 cm by:

$$I_m = 1000/N$$

Reducing compounds

Several reducing compounds could be detected: Oses and holosides and mucilages, by preparing an aqueous decoction from 10 g of powdered plant material in 100 ml of distilled water for 15 min.

After evaporating to dryness 5 ml of decoction, three drops of concentrated sulphuric acid were added followed by four drops of a saturated solution of thymol in ethanol. A red coloration indicated the presence of oses and holosides. Mucilages were detected by the appearance of a flocculent precipitate on adding 5 ml of pure ethanol to 15 ml of the decoction.

Cyanogenic heterosides are often present in plants. They were detected by suspending 1 g of powdered plant material in 5 ml of an equimolar solution of water and toluene. A reagent strip impregnated with Guignard reagent (2 g of picric acid and 20 g of sodium carbonate in 200 ml of distilled water) was then placed in the tube. A red coloration indicated the presence of cyanogenic heterosides.

The presence of coumarins was detected by evaporating to dryness 5 ml of the ether extract made for the detection of sterols and triterpenes). To this extract was added 2 ml of hot water followed by 1 ml of 25% ammonia. The mixture was examined under UV light at 366 nm. An intense blue fluorescence indicated their presence.

RESULTS

Ethnobotanical surveys

The ethnobotanical surveys revealed the different use of leaves, fruits, galls, mistletoes, roots and root barks for the treatment of various illness (Tables 1 to 5). The same part of the plant was used to treat various illness and at the same time different parts of the plant was used to treat a single illness (e.g. *Plasmodium falciparum* and pain). The leaves was the most used part in medicine followed by root and root barks. The fruits, galls and mistletoe were the least used medicinally plant parts. The method of preparation of medicine were mostly decoctions and macerations while powders were seldom

Table 1. Use of leafy branches from *G. senegalensis* in traditional medicine.

Illness treated	Treatment	Location
Plasmodium falciparum pernicious attack	Decoction of branches combined with leaves of *Momordica charantia* + 100 goat dung balls. Mixture drunk morning and evening for 2-3 days.	Ségué
Caries and dental abscess	Tea made from leaves of *G. senegalensis* combined with leaves of *Prosopis africana* used for mouthwash and exposure to hot vapours.	Daral
Conjunctivitis	Juice from crushed leaves instilled into the affected eye.	Ségué
Diarrhoea and dysentery	Decoction of leafy branches drunk morning and evening. Fresh leaves chewed and juice swallowed.	Ségué
	Washing every 2-3 days, morning and evening, with a decoction of leafy branches.	Badougou nafadji, Ségué
Aches and pains	Decoction or maceration of ground leaves used for washing, or drunk.	Daral
	Singed leafy branches covered with a cloth and placed in contact with painful area.	Daral
	Powdered leaves combined with karite butter used as an ointment.	Daral
Epilepsy	Maceration of ground leaves of *G. senegalensis* and *Pterocarpus erinaceus* combined with a cotyledon from a white cola nut in a new gourd, drunk or used for bathing for a long period.	Daral
	Maceration for 24 h of ground leaves from *G. senegalensis* combined with fruits of *Tamarindus indica* and honey. The solution obtained is drunk in the morning before breakfast for three days to treat malaria.	Ségué
Fever and malaria	Decoction of fresh leaves, drunk or used for bathing for three days to relieve fever in children.	Daral, Ségué
	Decoction of leafy branches, drunk to treat fever with vomiting.	Badougou nafadji
	Decoction of leaves of *G. senegalensis* combined with leaves of *Combretum glutinosum*, drunk or used for bathing to relieve fever. Decoction of leaves of *G. senegalensis* combined with leaves of *Combretum.*	Ségué
	Combretum micranthum, drunk or used for bathing to treat malaria.	Ségué
Sexual impotence	Decoction of leafy branches harvested on a Thursday at dusk, used to bathe and expose the penis to hot vapours.	Badougou nafadji
Unknown illness	Leafy branches harvested at dusk and brought home, making sure no fly has landed on them. Decoction when drunk will probably detect the illness.	Daral, Ségué
	For unknown or imprecisely known illness, decoction or maceration of leaves of *G. senegalensis* combined with leaves of *Parkia biglobosa* or with leaves of *Sclerocarya birrea* or with leaves of *Vitellaria paradoxa* or with leaves of *Kaya senegalensis,* drunk and used for bathing.	Daral

Table 1. Contd.

Headaches with fever	Decoction of leafy branches used to bathe and expose the patient's head to hot vapours.	Ségué
	Small red-hot stones are thrown into freshly ground leaves wrapped in a blanket and vapours inhaled.	Ségué
Abdominal pain	Three bundles of leafy branches are harvested on a Monday morning, with the gatherer's back turned to the plant. These are combined with three bundles of leafy branches of *Combretum micranthum* and unripe fruits of *Tamarindus indica.* Fresh mixture ground together and a decoction made, which is drunk.	Badougou nafadji, Ségué
	The decoction thus obtained also used to treat poisoning. Mixture contra-indicated in pregnant women, in whom it can induce abortion.	Badougou nafadji, Ségué
Migraine	A decoction is made from three bundles of leafy branches. A red-hot stone is added and the patient's head exposed to the hot vapours for seven days running.	Ségué
	Three or four bundles of leafy branches are collected. Three or four stones (depending on patient's sex) are heated. When red-hot the stones are placed on two of the bundles laid in an earthenware jar. One or two bundles are placed on top, followed by one or two more red-hot stones. Water is added and patient's head exposed to the hot vapours.	Ségué
Wounds	Fresh leaves are chewed and the resulting paste applied to fresh cuts. This stops the bleeding.	Ségué
	Wound washed with the decoction of the leaves. Young leaves then ground into a paste and used as a poultice on the wound. Healing properties.	Ségué

Table 2. Use of fruits from *G. senegalensis* in traditional medicine**.**

Illness treated	Treatment	Location
Uterine prolapse	When intercourse is obstructed, fruits of G*. senegalensis* are combined with the blood of an old hen (who has raised many offspring) and the vagina exposed to the hot vapours.	Daral
Abdominal pain	Fruits of *G. senegalensis* combined with fruits of *Gardenia ternifolia* and salt crystals. Mixture ground and ingested.	Badougou nafadji
Cough	Decoction of fruit is drunk. A small piece of crystallised salt can be added.	Badougou nafadji

employed. Treatments were mostly orally adminstered.

Analytical results

These results, given in Tables 6 to 12 are classified according to five observation criteria:

- Strongly positive reaction + + + +
- Positive reaction + + +
- Moderately positive reaction + +
- Weakly positive reaction +
- Negative reaction 0

The assay results for Ségué are given in Table 6; similar results were obtained from the two other sites (Badougou nafadji and Daral). The root barks contained a higher ethanol soluble extracts. There was no significant difference present in the various analyses for each plant part. The Mayer test indicated the presence of alkaloids

Table 3. Use of galls from *G. senegalensis* in traditional medicine.

Illness treated	Treatment	Location
Various illnesses	Decoction of galls of *G. senegalensis* drunk, used for bathing or enema used to treat many illnesses in children and infants: fever, malaria, vomiting, diarrhoea, irregular bowel movements, cough.	Ségué
Wounds	Galls ground and powdered, placed on the wound. Healing properties.	Ségué
Cough	Galls calcined, powdered with crystallised salt and ingested. Powder of 100 galls from 100 individual *G. senegalensis* plants ingested pure or incorporated into gruel. A decoction can be made of these galls and drunk. Galls of *G. senegalensis* combined with fresh fruits of *Tamarindus indica* and cystallised salt are powdered and ingested.	Ségué

Table 4. Use of mistletoe from *G. senegalensis* in traditional medicine.

Illness treated	Treatment	Location
P. falciparum pernicious attack	Decoction drunk or used as enema in children. The dried mistletoe can also be powdered and macerated to make an enema.	Ségué
Cataract	The leafy mistletoe combined with the leafy mistletoe of *Ficus capensis* is powdered and used to wash the patient's face on waking in the morning and in the evening before sleep.	Daral
Fever	Decoction generally used as an enema and for bathing in infants.	Ségué

Table 5. Use of root and root bark from *G. senegalensis* in traditional medicine.

Illness treated	Treatment	Location
Dental caries	Root used as toothpick for protection against dental caries.	Badougou nafadji, Daral
	Root powdered and macerated is used as a mouthwash morning and evening.	Daral
Diarrhoea and dysentery	Root bark combined with red sorgho and powdered is used to treat dysentery.	Ségué
	Roots macerated for three days, and solution mixed with honey and drunk for three days.	Ségué
Aches and pains	Root decoction used for bathing.	Ségué
Fever	Decoction of roots from plants found on roadsides is used to treat fever.	Ségué
	Decoction of roots drunk and used for bathing to treat night fever. Inhalation of vapours from decoction of fresh leaves for three days.	Ségué
Impotence	Root bark mixed with crystallised salt and fruits of *Aframomum melegueta*, powdered and ingested.	Daral
Abdominal pain	Decoction of roots from plants growing on anthills, drunk.	Ségué
Wounds	Powdered root bark placed on wound.	Ségué
Poliomyelitis	Decoction of roots harvested from midnight used to massage affected foot or feet, patient seated in the solution. Treatment repeated for at least one month. Each session to last 20-30 min.	Ségué

Table 6. Analytical results for different parts of *G. senegalensis*.

Assay	Ségué			
	Content (%)			
	Leaves	Fruits	Root bark	Stem bark
Water-extractible substances	9.0	9.0	9.0	9.0
Ethanol-extractible substances	5.1	7.3	31.2	12.4
Water: gravimetric method	5.9	6.9	5.0	6.3
Water: azeotropic method	6.0	6.0	6.0	6.0
Total ash	3.4	3.5	3.2	3.7
Sulphuric ash	7.8	7.5	12.2	2.5
HCl-insoluble ash	0.3	0.3	0.6	0.7

Table 7. Results of tests for alkaloids in different parts of *G. senegalensis*.

Site	Alkaloids			
	Leaves	Fruits	Root bark	Stem bark
Badougou Badougou nafadji	++	++	++	++
Daral	++	++	++	++
Ségué	++	++	++	++

Table 8. Results of tests for polyphenols in different parts of *G. senegalensis*.

Site		Tannins			
		Leaves	Fruits	Root bark	Stem bark
Badougou nafadji	Catechin	+++	+++	+++	+++
	Gallic	+++	+++	+++	+++
Daral	Catechin	+++	+++	+++	+++
	Gallic	+++	+++	+++	+++
Ségué	Catechin	+++	+++	+++	+++
	Gallic	+++	+++	+++	+++
Site		**Flavonoids**			
		Leaves	**Fruits**	**Root bark**	**Stem bark**
Badougou nafadji	Free flavonoids	+++	++	0	0
	Anthocyans	0	0	++	++
	Leucoanthocyans	0	0	0	++
Daral	Free flavonoids	++	++	++	++
	Anthocyans	0	0	+	+
	Leucoanthocyans	++	0	++	++
Ségué	Free flavonoids	++	++	0	0
	Anthocyans	0	0	0	0
	Leucoanthocyans	+++	+++	+++	+++

in leaves, root bark and fruits (Table 7). Plant parts from all three sites were rich in gallic, catechin tannins and free flavonoids (Table 8).

Anthocyans were only present in leaves and fruits as see in Table 9. Sterols, triterpenes and heterosides were present in each plant part for each of the three sites (Tables 10 and 11). The reducing compounds, oses and holosides were abundant in all plant parts fro each site

Table 9. Results of tests for anthracene derivatives in different parts of *G. senegalensis*.

Site	Free anthracene derivatives			
	Leaves	Fruits	Root bark	Stem bark
Badougou nafadji	+++	+++	0	0
Daral	+++	+++	+	+
Ségué	+	++	0	0

Table 10. Results of tests for sterols and triterpenes in different parts of *G. senegalensis*.

Site		Sterols and triterpenes			
		Leaves	Fruits	Root bark	Stem bark
Badougou nafadji	Lieberman reaction	+++	+++	+++	+++
	Carotenoids	0	0	0	0
Daral	Lieberman reaction	+++	+++	+++	+++
	Carotenoids	0	0	0	0
Ségué	Lieberman reaction	+++	+++	+++	+++
	Carotenoids	0	0	0	0

(Table 12).

DISCUSSION

Ethnobotanical investigation

The ethnobotanical survey conducted at the three locations Badougou nafadji, Daral and Ségué revealed common uses for *G. senegalensis* by practitioners of traditional medicine. To relieve aches and pains, and to treat fever and malaria, decoctions of leaves and leafy branches were prescribed at all three locations. These treatments are those already described in the literature for *G. senegalensis* (Koumaré, 1968; Fiot et al., 2004).

Roots were used as toothpicks to prevent dental caries at Badougou nafadji and Daral. Traditional medical practitioners at Badougou nafadji and Ségué prescribed using leafy branches combined with those of *C. micranthum* and unripe fruits of *Tamarindus indica* to relieve abdominal pain. The broadly ranging beneficial effects attributed to *G. senegalensis* make it suitable for the treatment of imperfectly diagnosed illnesses. It was used in this way at Daral and Ségué. We see that different plant parts are used to treat the same illness, although marked differences in treatment protocols were used. Thus leaves and galls were used at Ségué for their healing properties.

Cough was treated using a decoction of fruits at Badougou nafadji and a decoction or powder mixed with food at Ségué. For pernicious malarial attacks, decoctions of leaves or mistletoe were prescribed at Ségué. Severe diarrhoea and dysentery were countered by prescribing a decoction, or maceration of leaves or root bark at Ségué. At Ségué, traditional medical practitioners prescribed a decoction of root bark to relieve aches and pains, while at Daral, a decoction of leaves was prescribed for the same purpose. At Badougou nafadji, powdered fruit of *G. senegalensis* combined with fruit of *Gardenia ternifolia* was used for abdominal pains, while at Ségué, a decoction of roots was preferred. A decoction of leaves was used to treat sexual impotence at Badougou nafadji, while at Daral root bark was used. We note certain uses specific to each location. Thus galls were used by traditional medical practitioners only at Ségué, mistletoe was not prescribed at Badougou nafadji, and fruits were not used at Ségué. Interestingly, we recorded a broader variety of uses, often associated with chamanic practices, at Ségué. This observation may be partly due to better preserved ancestral practices in this region, which is more remote from a developed urban centre.

Discussion of analytical tests

The assay results for Ségué are given in Table 6.
Comparable results were obtained at Badougou nafadji and Daral. Water assay using the azeotropic or gravimetric method gave comparable results, the fruits containing marginally more water than the other parts. In all cases the water content was less than 10%. Hence these parts can be stored with no risk of enzymatic hydrolysis. These results are comparable to those

Table 11. Results of tests for cardiotonic heterosides in different parts of *G. senegalensis*.

Site	Cardenolides			
	Leaves	Fruits	Root bark	Stem bark
Badougou nafadji	+++	++	+++	+++
Daral	++	++	+++	+++
Ségué	+	+++	+++	+++

Table 12. Results of tests for other substances in different parts of *G. senegalensis*.

Site		Other substances			
		Leaves	Fruits	Root bark	Stem bark
Badougou nafadji	Reducing compounds	+++	+++	+++	+++
	Oses and holosides	++	++	++	++
	Mucilages	++	+++	++	++
	Coumarins	++	++	0	0
Daral	Reducing compounds	+++	+++	+++	+++
	Oses and holosides	+++	+++	+++	+++
	Mucilages	++	+++	++	++
	Coumarins	++	+	0	0
Ségué	Reducing compounds	+++	+++	+++	+++
	Oses and holosides	+++	+++	+++	+++
	Mucilages	++	+++	++	++
	Coumarins	++	++	0	0

Tests for combined anthracenosides, cyanogenic heterosides, and foaming index determination were all negative.

reported in the literature (Fiot et al., 2004).

The percentage of total ash from the four plant parts was relatively low, reflecting the small quantities of mineral matter present in these parts. As expected, the root bark contained a slightly greater non-volatile fraction. Ash insoluble in hydrochloric acid represents silicaceous plant matter. This fraction was very small; less than 1% in all four plant parts. These results were similar to those reported by Koumaré (1968). Sulphuric ash is a measure of the quantity of inorganic cations contained in the plant material. The leaves and fruits gave about 8%. The value for root bark was low, but was greater than 12% for stem bark. These results confirm the presence of alkaline earth metals (Mg, Ca), Fe, Ti, etc., as found by Koumaré (1968). The presence of Ca might account for the use of the plant to prevent dental caries. The presence of iron might explain its use against anaemia.

The percentage of water-extractible substances was the same for all four organs and equal to 9%. The 96% ethanol-extractible fraction was similar in leaves and fruits at about 6 and 7%, against 12% for the stem bark and 31% for the root bark. Alkaloids are present all parts of the plant. Three alkaloids had already been isolated and identified from leaves and roots: harman, tetrahydroharman and harmalan (Combier et al., 1977; Fiot et al., 2006). These alkaloids possess an antimalarial activity and a low cytoxicity, which might account for the prescription of decoctions of leaves to treat malaria (Ancolio et al., 2002). Total alkaloids extracted from leaves showed an antitussive activity (Faye et al., 1980; Diatta et al., 2007). The nature of the alkaloids present in the stem bark and fruits has not yet been studied.

Several studies of gallic and catechin tannins compounds have been published (Koumaré, 1968; Kerharo and Adam, 1974; Makar and Becker, 1994; Bouchet et al, 2000; Fiot et al., 2006; Sereme et al., 2008). The presence of tannins, in particular gallic tannins, might explain the use of *G. senegalensis* to treat respiratory tract disorders and cough (Neszmélyi et al., 1993; Bouchet et al., 2000). Certain gallic tannins possess an antidiarrhoeal activity, which might account for the use of *G. senegalensis* to treat severe diarrhoea and dysentery. It has been shown that 3,4,5-tri-O-galloylquinic acid isolated from *G. senegalensis* displays an anti-HIV activity (Mahmood et al., 1993).

Free flavonoids were present in leaves and fruits from all three sites, but they were present in root bark or stem bark only in plant material from Daral. This result is at variance with earlier literature reports (Bouchet, 1989; Ficarra et al., 1997; Maleset al., 1998; Bucar et al., 1998;

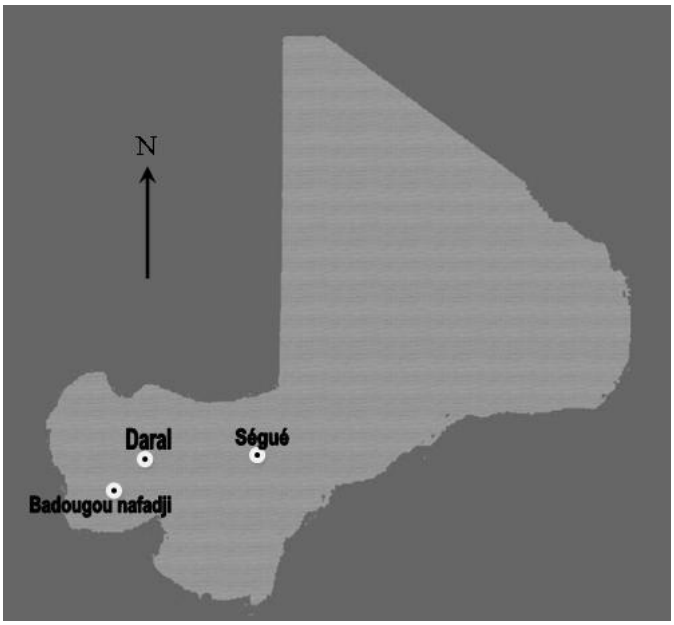

Figure 1. Map of Mali showing the different locations.

Fiot et al., 2006).

Anthocyans were present only in root and stem bark from Badougou nafadji and Daral. Leucoanthocyans were abundant in all plant parts from Ségué, less abundant in leaves, root bark and stem bark from Daral and present only in stem bark from Badougou nafadji. This disparity, in particular between Badougou nafadji and Daral on the one hand and Ségué on the other, shows the influence of climatic conditions and soil on the biosynthesis of these compounds. The existence of chemotypes of *G. senegalensis* is therefore probable.

Flavonoids occurred in different forms. These are known to have vascular protective and venotonic effects, although their utility has been challenged by the Food and Drug Administration (Bruneton, 1993).

Even so, this diverse occurrence of flavonoids in the plant parts might account for its use for the treatment of aches and pains and its venolymphatic effects. In addition, we note that the antiviral effects of flavonoids extracted from *G. senegalensis* against several DNA and RNA viruses have been described (Narayana et al., 2001).

Free anthracene derivatives were present only in leaves and fruits. We note that, just as for flavonoids, the Ségué location differed from Badougou nafadji and Daral by smaller amounts of these compounds.

Combined anthracene compounds were not found in any plant part from any of the three locations. This absence is consistent with the prescription of *G. senegalensis* to treat diarrhoea, as these substances are known to exhibit certain laxative properties (Bruneton, 1993).

Triterpenes and sterols were found in abundance in all plant parts, but no carotenoids were detected. These terpene derivatives, which most often possess marked biological activities, might justify the prescription of *G. senegalensis* by traditional medical practitioners to treat malaria and its pernicious attacks. Cardiotonic heterosides, or cardenolides, were present in all the plant parts from all three locations. However, the results were uneven. Once again, the Ségué differed from the two others by smaller amounts of cardenolides in leaves.

Although, all the plant organs formed some foam in aqueous solution, the foaming index was always nil, showing no saponosides were present. This result confirms the findings of Koumaré (1968), who report a nil foaming index for leaves and roots. Reducing compounds, oses and holosides were present in abundance in all plant parts from all three locations.

Mucilages were present in abundance, especially in fruits, but also in other plant parts from all three sites. The use of *G. senegalensis* to treat diarrhoea might also be due to the fact that mucilages are conducive to firmer stools, and so can avert sudden dehydration.

Coumarins, which are polyphenol compounds presenting anti-inflammatory, antimicrobial and anticoagulant properties, were present in only leaves and fruits. This might justify the use of the leaves of this plant by traditional medical practitioners at Ségué as an anticoagulant for the treatment of wounds. Lastly, the complete absence of cyanogenic heterosides was consistent with the non-toxicity claimed for *G. senegalensis* (Koumaré, 1968).

Conclusion

The ethnobotanical survey we conducted demonstrates the widespread and varied use (in terms of both indications and treatment protocols) of *G. senegalensis* by traditional medical practitioners. Several uses of different parts of *G. senegalensis* recorded in the course of our survey are newly reported. This is the case in particular for the use of decoctions of leaves to relieve abdominal pain and migraine by traditional medical practitioners at Ségué. The fruits were prescribed to treat uterine prolapse (Daral) by contact with vapours, and to treat cough by means of a decoction (Badougou nafadji), (Figure 1). Galls were used only at Ségué, to treat wounds and cough. At the same location a decoction of roots was prescribed to treat poliomyelitis.

The widespread use of leaves in different forms reflects the broad range of chemical substances present in them and the ease with which they can be collected.

The chemical analysis tests we carried out confirmed the results of earlier work (Koumaré, 1968; Fiot, 2004). In addition, we demonstrated the presence of certain chemical families that had not been studied hitherto: cardiotonic heterosides, sterols, triterpenes, oses and holosides, anthracene derivatives and anthocyans.

We found a degree of variability in the chemical

composition of plant parts according to the harvesting location. Thus while samples from Badougou nafadji and Daral appeared homogeneous, differences were noted in the material from Ségué where the climate is Sahelian. This might partly explain why uses of *G. senegalensis* by traditional medical practitioners of Ségué were sometimes different from those at the other locations. However, the importance of cultural factors in the use of the traditional pharmacopoeia must also be taken into account.

Thus, *G. senegalensis* is a plant with multiple pharmacodynamic activities that provides numerous popular remedies. Its activity profile remains to be described in full. Some of its properties, e.g. antidiarrhoeal and ulcer-protective (Aniagu, 2005), anti-inflammatory (Koumaré, 1968), antitussive (Koumaré, 1968; Faye et al., 1980; Pousset et al., 1983; Diatta et al., 2007) and hypertensive (Koumaré, 1968) have been demonstrated experimentally and may account for its inclusion in the West African pharmacopoeia.

Although several chemical constituents of *G. senegalensis* have been detected, isolated and characterised, e.g. the three alkaloids harman, tetrahydroharman and harmalan (Combier et al., 1977; Fiot et al., 2006), many of them have not yet been identified. The further investigation of the potentially active constituents of *G. senegalensis* is therefore an exciting challenge that may add to our phytotherapeutic arsenal.

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APPENDIX

List of plants cited in the present article.
Aframomum melegueta
Combretum aculeatum
Combretum glutinosum
Combretum micranthum
Ficus capensis
Gardenia ternifolia
Guiera senegalensis
Kaya senegalensis
Melanthera scandens
Momordica charantia
Parkia biglobosa
Prosopis Africana
Pterocarpus erinaceus
Sclerocarya birrea
Tamarindus indica
Vitellaria paradoxa