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# Physical characteristics and Chemical compositions of Leaves extracts of *Sorindeia grandifolia* Engl. (Anacardiaceae) harvested at Kato, Benin

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## Abstract

*The results brought back at the end of this work concern various chemical constituents of S. grandifolia leaves collected in Benin. The major compounds (≥ 4%) of essential oils obtained after hydrodistillation and analysis by coupling gas chromatography with spectrometry mass are constituted of : limonene (20.2%), (E)-β-ocimene (7.6-17.8%), (Z)-β-ocimene (11.4%), γ-cadinene (7.0%), selin-11-en-4-α-ol (4.5-5.7% ), palmitic acid (4.3-6.2%), β-pinene (5.5%), α-selinene (5.5%), α-phellandrene (5.2%), β-selinene (4.9%), β-elemene (4.5%).The lipidic fractions realized from petroleum ether extracts are marked by importants rates of arachidic acid (22.5%), palmitic acid (20.3%) and of linoleic acid (15.9 %). The phytochemical analysis showed relatively a large content of coumarins, gallic tannins, flavones, leucoanthocyans and saponins in opposition to the other secondary metabolites.*

**Keywords**: limonene, arachidic acid, metabolites, coumarins

## Introduction

Since ancient times, plant extracts, in particular aromatic, are sought for their bioactive properties. These plants are frequently used in the traditional pharmacopoeia in Benin for the therapeutic purposes in particular to relieve certain cutaneous affections, respiratory disorders, gastrointestinal diseases and cardiovascular etc. It is also attributed to the aromatic plants extracts likely biological properties of potential applications in modern medicine, food industry, perfumery and cosmetics: antiradical and anti-inflammatory powers[1].

*Sorindeia grandifolia* is an aromatic species of anacardiaceae family met in wooded savannas, dry forests, dense forests and humid regions of Western and Central Africa, situated in the North of the equator. This shrub, lianescent, bears leaves and supporting leaflets oblong and lanceolate, a pale green calyx, petal pink, white or pink flowers and fruits of ellipsoidal shape at maturity[2]. In Benin, the decoction of *S. grandifolia* leaves, consumed in the fresh state, treats the cough while its juice possesses antipyretic properties[3]. To date, the literature has not reported at least one bioactive chemical compounds in this plant whose therapeutic performance in traditional medicine has always proven. This work has for objective the valorization of this species (*Sorindeia grandifolia* Engl., Anacardiaceae) from Benin by the extraction, the identification and the characterization of bioactive compounds it contains. The purpose of this study is to determine, through appropriate analytical methods, the physicochemical characteristics of this plant leaf extracts.

## Material and Methods

**Plant material and distillation of the volatile constituents:** *Sorindeia grandifolia* leaves were collected in June and July 2007 in the classified forest of Lama (Benin). They were authenticated at the National Herbarium of the University of Abomey-Calavi. In the laboratory, these leaves are kept between 18 and 20°C in the shade of sunlight throughout the study period. Essential oil is extracted from leaves (450g) by the technique of hydrodistillation during five hours on Clevenger, according to the method used in british pharmacopoeia[4]. They was dried over anhydrous sodium sulfate and analyzed by GC/FID and GC/MS. For the determination of non-volatile compounds, powders were obtained from the leaves, dried in the dark for one month, by grinding with a knives machine Ika Werke MF 10 basic type. Vegetable powders collected are then sieved in the size grading 0.425.

**Physical properties:** Physical parameters of the essential oil of *S. grandifolia* leaves were determined using the methods described by AFNOR[5,6].

**Density at 20°C:** The density measure was carried out using a micro-pycnometer and a precision balance.

**Refractive index at 20°C :** The refractive index was determined by means of the refractometer CARL ZEISS JENA 234678.

**Rotatory power at 20°C:** The measurement was made by Carl Zeiss polarimeter 128291.

**Phytochemical analysis: Analysis of the volatile constituents: GC/FID:** The extracts were analysed on a Hewlett-Packard gas chromatograph Model 6890, equiped with a DB5 MS column (30 m x 0.25 mm, 0.25 µm), programming from 50°C (5 min) to 300°C at 5°C/min, 5 min hold. Hydrogen as carrier gas (1.0 mL/min); injection in split mode (1:60); injector and detector temperature: 280 and 300°C respectively. Each extract is diluted in hexane: 1/30.

**GC/MS**: The extracts compositions were analysed on a Hewlett-Packard gas chromatograph Model 5890, coupled to a Hewlett-Packad MS model 5871, equipped with a DB5 MS column (30 m x 0.25 mm, 0.25 µm), programming from 50°C (5 min) to 300°C at 5°C/min, 5 min hold. Helium as carrier gas (1.0 mL/min); injection in split mode (1:30); injector and detector temperature, 250 and 280°C respectively. The MS working in electron impact mode at 70 eV; electronmultiplier: 2500 eV; ion source temperature: 180°C; mass spectra data were acquired in the scan mode in *m/z* range 33-450.

The compounds assayed by GC in the different essential oils were identified by comparing their retention indices with those of reference compounds in the literature and confirmed by GC-MS by comparison of their mass spectra with those of reference substances[7,8,9].

**Analysis of non-volatile constituents of leaves of *S. Grandifolia:*** The phytochemical screening was made according to the standard techniques described by Paris and Moyse[10], Bouquet[11] and Debray *et al*.[12].

Coumarins: Infused at a 10% made from the plant powder was examined under the UV light (365 nm). The appearance of a bluish fluorescence indicated a positive reaction.

Tannins gallic: An aqueous infusion was prepared from 5 g of plant powder and 100 mL of boiling distilled water. After 15 min, the mixture was filtered. The residue was rinsed with hot water to bring the volume of the filtrate to 100 mL. 20 mL of the filtrate are saturated with sprayed sodium sulfate and then, it was added dropwise 1 mL of ferric chloride (1%).The development of a blue-black tint corresponded to the presence of gallic tannins, not precipitated by Stiasny's reagent.

Flavones: It was introduced in a test tube, 5 mL of infused. In this content, 5 mL of hydrochloric alcohol constituted by equal volumes of ethanol at 95°, distilled water, concentrated hydrochloric acid (37%) and 1 mL of isoamylic alcohol were added. In the presence of shavings magnesium, it emerged at the supernatant layer (layer isoamyl alcohol) a pink-orangy color indicating the presence of genins of flavonoids.

Leucoanthocyans: They were identified by introducing into a test tube 5 mL of infused (5%) and 5 mL of hydrochloric (ethanol 95 ° + distilled water + hydrochloric acid 37% of equal volumes). The mixture was compete with 1 mL of isoamylic alcohol and then, heated to 90° through a water bath. After fifteen minutes, it had developed a red-cerise tint (or purple) indicating the presence of leucoanthocyans.

Saponins: A decoction was prepared during 30 min from two grams of plant powder and 100 mL of distilled water. After filtered the obtained mixture, the filtrate was divided into 10 different volumes (1 mL, 2 mL, 3 mL, 10 mL) in 10 calibrated tubes (internal diameter :1.3 cm). The content of each tube was adjusted to 10 mL with distilled water. After shaking each tube in a horizontal position for 15 seconds, followed by a rest of 15 min in an upright position, the height of the foam supernatant was measured in cm. When this height is close to 1 cm in the $X^{th}$ tube, the foam index (I) is calculated by the following formula: I = Foam height (in cm) in the $X^{th}$ tube × 5/0. 0X. The presence of saponins in the plant is confirmed when the value of the foam index is greater than 100.

**Fatty acids (FA) and unsaponifiable (Un):** Extraction of lipids: 15.0 g of the vegetable material powder were twice extracted successively by 100 mL of petroleum ether (40-60°C) with magnetic stirring at room temperature. After filtration and evaporation of the solvent under reduced pressure, the extracts were dried and weighed. The yields were established in calculating the average of three extractions.

Saponification and fatty acids obtention: The saponification was conducted by refluxing, during thirty minutes past one o'clock, 0.5 g of plant extract and 25 mL of an ethanolic and potassium hydroxide solution (2N). After cooling, there was added 50 mL of water and the unsaponifiable matter are extracted by 3 × 50 mL of cyclohexane. The soap solution produced was then acidified to precipitate the FA (5 ≤pH≤ 6). The FA released were yet extracted by 3 × 50 mL of diethyl ether[13,14].

Methylation of FA: Fatty acids were converted to their methylic esters by addition a methanolic solution (10%) of boron trifluoride ($BF_3$) and the methylic esters were extracted with cyclohexane.

The analysis of FA and Un collected was made through GC/FID and GC/MS[15].

## Results and Discussion

The measurement of density, refractive index and rotatory power of the essential oils samples of *S. grandifolia* leaves collected at different dates to Kato (the Lama botanical forest reserve of Benin) gave the values included in the table 1.

**Table-1**
**Physical characteristics of the essential oils of *Sorindeia grandifolia***

| | **Density (20°C)** | **Refractive index (20°C)** | **rotatory power (20°C)** |
|---|---|---|---|
| $L_1$ | 0.917 | 1.4935 | -7.996 |
| $L_2$ | 0.921 | 1.4932 | 5.000 |
| $L_1$ = Leaves (07-06-07) ; $L_2$ = Leaves (07-07-07) | | | |

The values determined for the density and the rotatory power conversely varies to those of the refractive index after a month of harvest interval of $L_1$ and $L_2$. According to these values, there is a slight difference between the densities of essentials oils extracted from *S. grandifolia* leaves. Between the values of refractive indexes, this difference is more weak. By cons, the difference between the values of the rotatory power is very pronounced. These differences noted could be bound to several factors, in particular, edaphic, climatic as well as the cultural practices[16,17,18]. It is important to appreciate in the future such homogeneity over several months.

*S. grandifolia* is a little fragrant species. The yields in essential oil of the leaves of this plant are low ($1.98x10^{-2}$% to 2. $17x10^{-2}$%). The values of these yields are seventeen for eighteen times lower than the value of the yield in essential oil of *Diplolophium africanum* stem-foliage reported by Koudoro *et al*. in 2011[19]. On the other hand, the *S. grandifolia* leaves seem richer in volatile extracts contrary to those of *Anona senegalensis* ($1.4x10^{-2}$%) investigated by Noudogbessi *et al.* in 2011[20]. The volatile compounds identified in the essential oils collected by hydrodistillation of leaves are presented in table 2.

GC-FID and GC- MS analysis of *S. grandifolia* essential oil revealed 40 compounds in the sample $L_1$ and 46 in that of $L_2$ representing respectively 96.9 and 94.1% of the total weight of the essential oil (table 2). The main families volatile of compounds which marking $L_1$ and $L_2$ are the hydrogenated monoterpenes (31.3 to 57.9%), hydrogenated sesquiterpenes (15.3% to 41.5%) and oxygenated sesquiterpenes (11.7 to 13.6%). The proportions by oxygenated monoterpenes no neighborhood that 3.5% in $L_1$ and 2.3% in $L_2$. The main constituents (≥ 4%) susceptible to generate a bioactive character in $L_1$ are (Z)-β-ocimene (11.4%), (E)-β-ocimene (7.6%), γ-cadinene (7.0%), selin-11-en-4-α-ol (5.7%), palmitic acid (6.2%), α-selinene (5.5%), α-phellandrene (5.2%), β-selinene (4.9%) and β-elemene (4.5%). The major compounds (≥ 4%) of $L_2$ volatile extract were constitued of limonene (20.2%), (E)-β-ocimene (17.8%), β-pinene (5.5%), selin-11-en-4-α-ol (4.5%) and palmitic acid (4.3%). Further phytochemical investigations on various extracts realized with appropriate solvents allowed indicate the presence in *S. grandifolia* leaves of other metabolites, proven pharmacological properties[21]. This is coumarin, gallic tannins, flavones, leucoanthocyans and saponins (table 3). The presence of saponins, assessed by calculating the foam index (83.33), would explain the plant high aphrogene power. It was also noticed traces of carotenoids, catechic tannins and free quinone.

The lipid content (specifically FA) obtained by quantitation after extraction with petroleum ether was estimated at 0.7% compared to the mass of plant material used.

FA identified by gas chromatography coupled with spectrometry mass are predominantly saturated (44.2%). As for the unsaturated FA, they represent only 15.9%. The major FA identified in *S. grandifolia* leaves are arachidic acid (22.5%), palmitic acid (20.3%), linoleic acid (15.9%), followed by traces of myristic acid (1.4%). The investigations led on the unsaponifiables did not reveal an interesting chemical composition. Indeed the compounds constituting the footsteps of Un collected could not be identified by GC/MS.

## Conclusion

The chromatographic studies of *S. grandifolia* leaves extracts showed the presence of high proportions of monoterpenes and sesquiterpenes hydrogenated in essential oils and FA often found in the vegetable kingdom. The phytochemical screening, performed on the basis of specific tests allowed to characterize in *S. grandifolia* leaves: coumarins, gallic tannins, flavones and leucoanthocyans. The phytochemical heritage of the *S. grandifolia* plant, omen, because it is referenced by traditional medicine in Benin, a useful contribution in the development of the vegetable biotechnology. These preliminary results will be used to support a rigorous exploration of pharmacological properties of the endemic species *S. grandifolia* of Benin.

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**Table-2**
**Chemical compositions of essential oils from the leaves of *S. grandifolia***

| Compounds | $KI_{exp}$ | $KI_{th}$ | (%) | |
|---|---|---|---|---|
| | | | $L_1$ | $L_2$ |
| *trans*-hex-2-enal | 848 | 846 | 0.4 | 1.1 |
| *trans*-hex-2-en-1-ol | 858 | 854 | - | 0.6 |
| hexan-1-ol | 867 | 863 | - | 0.3 |
| tricyclene | 925 | 921 | 2.5 | - |
| α-pinene | 936 | 932 | - | 2.5 |
| **β-pinene** | **978** | **974** | 3.3 | **5.5** |
| myrcene | 991 | 988 | 0.5 | 1.2 |
| meta-mentha-1(7),8-diene | 1002 | 1000 | - | 0.3 |
| **α-phellandrene** | **1004** | **1002** | **5.2** | 2.7 |
| p-cymene | 1022 | 1020 | - | 3.2 |
| o-cymene | 1026 | 1022 | - | 0.5 |
| **limonene** | **1029** | 1024 | 0.4 | **20.2** |
| **(Z)-β-ocimene** | **1037** | **1032** | **11.4** | 0.4 |
| **(E)-β-ocimene** | **1046** | **1044** | **7.6** | **17.8** |
| terpinolene | 1090 | 1086 | - | 3.1 |
| p-cymenene | 1092 | 1089 | - | 0.5 |
| linalool | 1098 | 1095 | 1.7 | 0.4 |
| p-cymen-8-ol | 1184 | 1179 | - | 0.8 |
| α-terpineol | 1191 | 1186 | 1.4 | 1.1 |
| geraniol | 1254 | 1249 | 0.4 | - |
| *trans*-verbenyl acetate | 1295 | 1291 | - | 0.3 |
| α-longipinene | 1354 | 1350 | 1.3 | - |
| α-ylangene | 1377 | 1373 | 1.3 | - |
| α-copaene | 1379 | 1974 | 1.1 | 0.5 |
| **β-elemene** | **1394** | **1389** | **4.5** | 2.2 |
| β-longipinene | 1405 | 1400 | 1.8 | 0.2 |
| β-caryophyllene | 1421 | 1417 | 3.2 | 1.7 |
| *cis*-thujopsene | 1432 | 1429 | 1.3 | 0.3 |
| β-copaene | 1433 | 1430 | - | 0.2 |
| selina-4(15),6-diene | 1447 | - | 1.6 | - |
| α-humulene | 1454 | 1452 | 1.4 | t |
| selina-4,11-diene | 1471 | - | 2.0 | 1.1 |
| γ-muurolene | 1480 | 1478 | 0.7 | 0.5 |
| **β-selinene** | **1492** | **1489** | **4.9** | 3.6 |
| **α-selinene** | **1501** | **1498** | **5.5** | 2.7 |
| α-bulnesene | 1512 | 1509 | 1.2 | 0.5 |
| **γ-cadinene** | **1515** | **1513** | **7.0** | 1.1 |
| δ-cadinene | 1524 | 1522 | 1.5 | 0.7 |
| *trans*-calamenene | 1530 | 1528 | 1.2 | - |
| spathulenol | 1579 | 1577 | 0.5 | 0.5 |
| caryophyllene oxide | 1584 | 1582 | 1.2 | 1.1 |
| globulol | 1593 | 1590 | - | 0.3 |
| humulene epoxide II | 1611 | 1608 | 0.7 | 0.6 |
| 1,10-di-epi-cubenol | 1622 | 1618 | 0.5 | 0.2 |
| citronellyl pentanoate | 1628 | 1624 | - | 0.3 |
| 1-epi-cubenol | 1632 | 1627 | - | 0.2 |
| epi-α-cubenol | 1640 | - | - | 1.1 |
| epi-α-muurolol | 1645 | 1640 | 1.1 | - |

| | | | | |
|---|---|---|---|---|
| α-muurolol | 1649 | 1644 | 0.5 | - |
| cubenol | 1651 | 1645 | - | 0.4 |
| α-cadinol | 1658 | 1652 | 1.9 | 1.7 |
| **selin-11-en-4-α-ol** | **1664** | **1658** | **5.7** | **4.5** |
| β-sinensal | 1704 | 1699 | - | 0.6 |
| (2Z, 6E)-farnesol | 1718 | 1722 | 1.0 | 0.5 |
| iso-longifolol | 1723 | 1728 | 0.5 | - |
| **palmitic acid** | **1967** | **1959** | **6.2** | **4.3** |
| oleic acid | 2148 | 2141 | 0.8 | - |
| **Monoterpene hydrocarbons** | | | **31.3** | **57.9** |
| **Oxygenated monoterpenes** | | | **3.5** | **2.3** |
| **Sesquiterpene hydrocarbons** | | | **41.5** | **15.3** |
| **Oxygenated sesquiterpenes** | | | **13.6** | **11.7** |
| **Total** | | | **96.9** | **94.1** |
| **$L_1$ = Leaves (07-06-07) ; $L_2$ = Leaves (07-07-07)** | | | | |

**Table-3**
**Chemical families identified in the leaves of *Sorindeia grandifolia***

| Coumarin | Gallic tannins | Flavones | Leucoant hocyanes | Saponins (foam index) |
|---|---|---|---|---|
| +++ | +++ | +++ | +++ | ++ (83.33) |
| **+++ : Abundant** ; **++ : Average** | | | | |

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