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# Study on the biological activities and chemicals compositions of *Vitex doniana* leaves extracts

# *Étude sur les activités biologiques et les compositions chimiques des extraits de feuilles de Vitex doniana*

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**Résumé**

***Contexte et objectif***. Bien que beaucoup de plantes soient bien connues avoir des propriétés antioxydantes, l'analyse ces propriétés des extraits de feuilles congolaises reste fragmentaire. L'objectif de la présente étude était de faire les analyses phytochimiques et d'évaluer des propriétés antioxydantes des extraits de feuilles de *Vitex doniana*, utilisées dans le traitement de diverses maladies. ***Méthodes***. L'huile essentielle a été obtenue par la distillation. Les extraits aqueux, eau - méthanol et d'huile essentielle ont été étudiés. Les composés phytochimiques antioxydants ont été déterminés, par HPLC. Les propriétés antimicrobiennes étaient évaluées sur l'extrait d'eau (WE) et l'extrait Méthanol/ eau (70%) (MWE). ***Résultats***. Les composés phénoliques ci-après ont été identifiés : la rutine (11943 µg/ml), la quercetine (1831 µg/ml), l'acide gallique (471,4 µg/ml) et l'homoorientine (384,1 µg/ml). Les principaux composés trouvés dans l'huile essentielle par GC/MS étaient l'acide margarique (25,88%), l'alcool myristique (18,42%), l'asaron (7,81%), le scytalon (3,32%), l'hyperforine (2,55%), le linalol (1,91%). Les analyses ont montré que l'extrait (Méthanol/eau) a les meilleures activités sur les germes suivants : *Escherichia coli*, *Aspergillus niger*, *Pseudomonas aeruginosa*; *Bacillus subtillis*, *Staphylococcus aureus*. ***Conclusion***. Ces résultats prouvent le potentiel antioxydant des extraits des feuilles de *Vitex doniana*, affirmant ainsi les propriétés thérapeutiques de cette plante utilisée par des tradipraticiens. Les feuilles de cette plante peuvent ainsi être recommandées pour une possible utilisation dans la biotechnologie.

**Mot clés** : Composés phénoliques ; composés phyto-chimiques ; huile essentielle ; RP-HPLC
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**Summary**

***Context and objective***. Little is known about the antioxidant property of Congolese plants. The present study aimed to realize a phytochemical screening and to assess antioxidant property of fresh leaves of *Vitex doniana*, used in the treatment of various diseases. ***Methods***. The essential oil was obtained by distilled method. The extracts (methanol, water and essential oil) were screened for the presence of phenolic compounds by RP-HPLC and GC/MS. ***Results***. Several phenolic compounds were identified such usrutin (11943 µg/ml), quercetin (1831 µg/ml), gallic acid (471.4 µg/ml), and homoorientin (384.1 µg/ml). The major compounds found in the essential oil were margaric acid (25.88%), Myristic alcohol (18.42%), asaron (7.81%), scytalon (3.32%), hyperforin (2.55%), linalool (1.91%). The antimicrobial properties evaluated on Water extract (WE) and methanol water extract (MWE) showed that MWE has better activities on *Escherichia coli*, *Aspergillus niger*, *Pseudomonas aeruginosa*; *Bacillus subtillis*, *Staphylococcus aureus*. ***Conclusion***. These results confirm the antioxidant potential of these extracts and likely the therapeutic effects. Therefore, *V. doniania* should be considered as a promising source of useful drugs.

**Keywords**: Polyphenolic compounds, phytochemical, essential oils, RP-HPLC

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## Introduction

Medicinal plants are rich bioresources of drugs. A number of interesting outcomes have been found with the use of extracts of natural products from plants to treat several diseases. The antimicrobial properties of plants have been investigated by researches in the world. Biological evaluation of the plants extracts is vital to ensure their efficacy. The use of the plants and plants derived products is more and more of alternative therapies (1).

Several papers have been published for pharmacological proprieties of medicinal plants or isolated constituents for their properties like antioxidant, antidiabetic, antibacterial, antiviral and antiulcer activities (2).

Plants parts (fruits, vegetables, medicinal herbs…) contain a wide variety of free radical scavenging molecules such as phenolic compounds (phenolic acids, flavonoids, anthocyannins and tannins), vitamins and some other metabolites which are rich in antioxidant activity (3-5). In previous studies, we have reported the evaluation of the antioxidant potential of some medicinal, essential oils and dietary plants and the positive correlation between peripheral blood granulocyte oxidative status (6).

Vitex doniania is a plant of Verbanaceae family, the tree grows in open woodland and savannah regions of tropical Africa. The Vitex doniania is commonest of Vitex species in West Africa. It produces fruits with are plum-like, sweet and edible (1). But, little is known about the antioxidant potential of the Vitex doniania. Therefore, the present study aimed to evaluate the level of total phenolics compounds in the essential oil from the leaves of Vitex doniania. The antioxidant potential was also assessed by using vitamin C equivalent capacity test (VEAC).

**Methods**

*Apparatus*

The RP-HPLC analyses were performed with a waters 600E pump coupled to a waters 486 UV visible tunable detector and equipped with a AltechIntersil ODS Column (RP CP C18 Column size 4.6 mm × 150 mm; particle size, 5 µm.

*Chemicals and bacterial strains*

Folin-Ciocalteu's phenol reagent, aluminum chloride catechin, gallic acid, p-coumaric acid, coumarin, rutin, chlorogenic acid, vitamin acid, delphinidin, orietin, malvidin, homoorientin, ellargic acid, l-cyanidin, peonidin, homoorientin, ellagic acid, l-cyanidin, peonidin were purchased from Across Organics. Sodium carbonate, sodium nitrite, chlorhydric acid, ethyl acetate, soduim sulfate anhydrous, ammonium phosphate, ferric ammonium sulfate, acetoninitrile, methanol 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic) (ABTS), PBS buffer, AAPH [2,2'-azino-bis (2, amidino-propane) dihydrochloride] and DPPH (2,2-diphenyl-1picrylhydrazyl) were obtained from Sigma and Roth (France). The chemicals used were all of analytical grade. For Antimicrobial properties, The following bacterial strains were employed in the screening: *Staphylococcus aureus* (ATCC29213), *Bacillus subtilis* (ATCC6059), *Escherichia coli* (ATCC25922), *Pseudomonas aeruginos* (ATCC6059) and fungi: *Aspergillus niger* (135550/99), *Candida albicans* (ATCC90028) (Lorraine University, Laboratoire IBISI Thionville).

*Producent and preparation of samples*

Fresh leaves of *Vitex doniana* plants were collected in 2016 from Kisantu (DR Congo). The leaves were identified at the laboratory of Botanics faculty of Sciences University of Kinshasa. All leaves materials were dried at room temperature and were ground and sifted in a sieve (0.75 µm).

Phenolic compounds extraction for RP- HPLC analysis

Polyphenolic were extracted with water (WE) and methanol –water (50/50 v/v) (MWE) as solvent according to the slightly modified method described by (4).

A sample (800 mg) was extracted with 2 x 10 ml of solvent under intermittent shaking (2 minutes) on vortex mixer for 30 min. The sample was centrifuged at 1536 xgr for 20 min at 20 °C. The supernant was taken into a 10 ml volumetric flask. The extracts were stored at 4°C until analysis.

Protocol: 3 mg of the residue from methanol-water extracts were chromatographed on a column 3cm in diameter and 50cm long containing 100g of silica (60-200µ). A gradient elution Dicloro Methane/ methanol (DCM / MeOH) was established, (n-Hexane, DCM, MeOH / DCM, which varies from 1% to 50% by volume) to separate the different phenolic

compounds according to their polarity. Extracted sample was filtered through a 0.45µm PTFE syringe tip filter. The flow rate was set at 1ml / minute at room temperature. A gradient of three mobile phases was used in the present study, solvent A: 50 mM ammonium phosphate ($NH_4H_2PO_4$) pH 2.6 (Adjusted with phosphoric acid); solvent B: Which was constituted of 80: 20 (v/v) acetonitrile / solvent A, and solvent C, constituted of 200 mM phosphoric acid pH 1.5 (pH adjusted with ammonium hydroxide). The solvents were filtered through a Whatman Maidstone England paper N° 113 and putted in an ultrasonic apparatus for 25 minutes.

The gradient profile for 60 minutes was: 100% solvent A at zero minutes, 92 % A / 8 % B at 4 minutes, 14 % B / 86% C at10 minutes, 16 %B / 84 % C at 22.5 minutes, 25% B /75% C at 27.5 minutes, 80% B / 20% C at 50 minutes, 100% A at 55 minutes, 100% A at 60 minutes. After each run, the system was reconditioned for 10 minutes before analysis of next sample. Under these conditions, 20 µl of sample were injected. All sample analysis was done in triplicate. Phenolic standards prepared by dissolving 1mg/ ml were used to generate characteristic UV spectra and calibration curves. Individual phenols in the sample were identified by comparison of their UV-Vis spectra and retention times with spiked in put of the corresponding phenolic standards.

The detection was carried out at 280 and 320 nm and their quantification was obtained by the comparison of the peaks areas with the corresponding standards calibration curves. Collected results were reported as equivalent amount of commercial standard. The retention times of the isolated phenolic compounds were compared to the corresponding standards. Some compounds were not identified because of lack of standard equivalents.

Extraction and isolation of *Vitex doniana* essential oil

* Hydro-distillation

Two hundred and fifty grams of the air-dried leaves of Vitex doniania (figure 1) was subjected to hydro-distillation for 3 h using a Clevenger type apparatus. Sodium chloride (1 g) and 20 mL of dichloromethane was added with the aqueous distillate in a separating funnel and shaking was continued for 40 min and allowed to stand for 15 min. The organic layer was separated and concentrated to 5 mL under reduced pressure. The oils dissolved in the organic layer were dried over anhydrous sodium sulphate and preserved in a sealed vial at 4°C until further analysis. The hydrodistillate oil was subjected to GC–MS.

**Figure 1**. Vitex doniania with fruit

* Analysis of the essential oils

The essential oils were analysed and identified by GC/ MS. An HP model 6890 GC equipped with a 30 m × 0.25 mm i.d. (df: 0.25µ m) DB-5 boded-phase fused-silica capillary column (Agilent, Folsom, CA) and a flame ionization detector (FID) were used. Injector and temperatures were 200 and 300°C, respectively. The oven temperature was programmed from 35 to 250°C at 5°C/ min and held for 50 min. The linear velocity of the helium carrier gas was 30 cm/s. Injections were in the spitless mode. An HP model 6890 GC interfaced to an HP 5791A mass selective detector (GC/MS) was used for mass spectral identification of the GC components at a MS ionization voltage of 70 eV. A 30 × 0.25 mm i.d (df= 0.25µ m) DB wax bonded-phase fused-silica capillary (Agilent, Folsom, CA) was used for GC. The linear velocity of the helium carried gas was 30 cm/s. The temperature of the injector and detector was 250°C. The oven temperature was programmed

from 50°C to 250°C at 5°C / min and held for 50°C. The percentage of each compound in the oil is determined from peak areas without correction factors account assuming that all components have coefficients of neighboring rethinking. Identifications of constituents were performed by coupling an HP model 6890 GC gas to a mass spectrometer type an HP 5791A mass selective detector (GC-MS). The volume injected is 1µ l of a pure oil solution diluted to 1% in dichloromethane. Quantitative analysis was based on the comparison of retention times and the computer mass spectra libraries using Wiley GC/MS Library and Nist, Tutore Libraries. The percentage composition was computed from the GC peak areas.

*Statistical analysis*

Results are presented as Mean ± Standard deviation; statistical analysis of experimental result was based on analysis of variance one way ANOVA. Significant difference was statistically considered at the level of p value ≤ 0.001.

## Results

*Qualitative and quantitative analysis of phenolic compounds by* RP-HPLC

Figures 2 and 3 show RP-HPLC chromatogram of methanol-Water extract (MWE) and Water extract (WE) respectively. Peaks were identified and quantified on the basis of their retention time values and uv spectra by comparison with those of single compound in the standard solution. The retention time and the concentration of phenolic compounds contained in the extracts are listed in table 1. Some numerous peaks were not identified because of suitable standards. The samples were analyzed at least four replications at 280 and 320 nm.

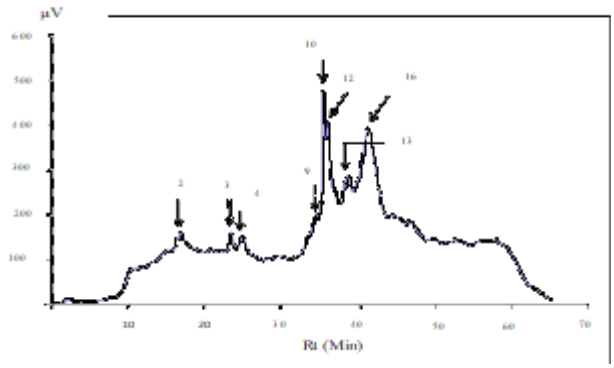


**Figure 2**. WE (280 nm) *V. doniana*, Chromatographic profile of Vitexdoniana
2: protocatéchuic acid; 3: catéchin acid; 4: chlorogénic acid; 9: p-coumaric acid; 10: homoorientin, 12: rutin; 13: G-quercétine; 16: cinnamicacid

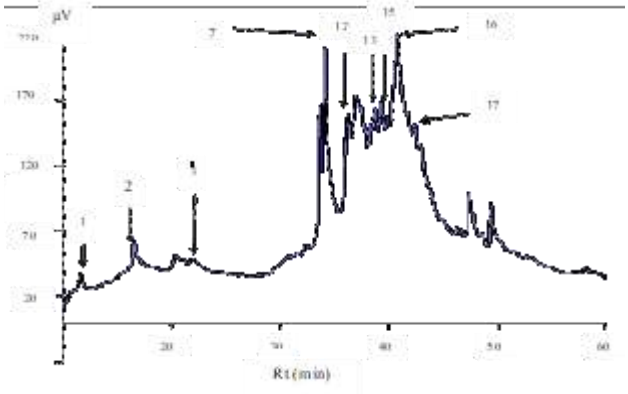


**Figure 3**. MWE (320 nm): *V. doniana*, Chromatographic profile of *Vitex doniana*
1 : Gallic acid ; 2 : protocatechuic acid ; 3 : catechine ; 7 : p-coumaric acid; 12 : rutine ; 13 : G-quercetin ; 15 : H-quercetin ; 16 : cinnamic ; 17 : quercetin

**Table 1. *Vitex doniana* extract phenolic compounds**

| Compound name | Rt (min) | $E_F$ (µg/ml) |
|---|---|---|
| gallicacid | 11.2 | 471.4 ± 0.2 |
| Protocatechiucacid | 17.0 | 34.8 ± 0.8 |
| catechin | 25.0 | 1.4 ± 0.1 |
| chlorogenicacid | 26.5 | 1.7 ± 0.1 |
| caféicacid | 28.7 | nd |
| *p*-coumaricacid | 33.4 | 18.8 ± 0.3 |
| homoorientin | 35.2 | 384.1 ± 2.0 |
| rutin | 37.1 | 11943 ± 5.0 |
| G-quercetin | 38.0 | 12.6 ± 1.0 |
| dH-quercetin | 39.3 | 1.7 ± 0.1 |
| cinnamicacid | 41.3 | nd |
| quercetin | 42.6 | 1831 ± 18.0 |

*Chemical compounds identified in Vitex doniana essential oils* (EO) *extract*

Yields of E.O from *V. doniana* leaves were 0.45 ± 0.02 g/100g of dry leaves (w/w). The value is the mean of standard deviation (n=3) ($p \leq 0.02$). Quantitative and qualitative results are shown in the table 2.

**Table 2 : *Vitex doniana* essential oils chemical-compounds**

| Ref | Rt (min) | Compound name | (% v/v (ml)) |
|---|---|---|---|
| 1 | 3.08 | 2-pentyl furan | 0.41 |
| 2 | 3.89 | , 3- hexen,1-ol | 1.55 |
| 3 | 7.06 | 6-méthyl, 5-hepten, 2-one | 0.70 |
| 4 | 7.55 | octanal | 0.72 |
| 5 | 7.79 | 2,4-heptadiènal | 0.53 |
| 6 | 9.75 | 1-methyl 3- (1"-methylcyclopropyl) cyclopentène | 0.40 |
| 7 | 10.38 | geraniol | 2.02 |
| 8 | 10.52 | pelargonaldehyde | 2.10 |
| 9 | 11.94 | 2,6-nonadienal | 0.50 |
| 10 | 12.15 | 2- nonenal | 0.60 |
| 11 | 13.50 | décanal | 0.70 |
| 12 | 13.88 | 2, 6,6 trimethyl cyclohexene-1carboxaldehyde | 0.50 |
| 13 | 15.09 | 3-heptyl acrolein | 0.65 |
| 14 | 15.37 | pelargonic acid | 0.68 |
| 15 | 15.62 | 1, 5,5 –triméthyl, 6-methylene cyclohexen | 0.73 |
| 16 | 15.86 | α-tocotrienol | 0.41 |
| 17 | 16.14 | theaspiran | 1.28 |
| 18 | 17.88 | 2-undecenal | 0.65 |
| 19 | 17.96 | capric acid | 0.41 |
| 20 | 18.22 | dehydroisophytole | 0.37 |
| 21 | 18.29 | damascenone (rose cetone) | 0.47 |
| 22 | 19.39 | α-terpinolene | 2.54 |
| 23 | 20.03 | linalool | 1.91 |
| 24 | 20.29 | α-caryophyllene | 0.53 |
| 25 | 21.35 | δ-cadinene | 0.45 |
| 26 | 21.77 | α-terpinèn | 0.40 |
| 27 | 22.90 | oleic acid | 2.11 |
| 28 | 23.38 | oxide caryophyllene | 0.53 |
| 29 | 23.53 | 3- tetradecanone | 0.74 |
| 30 | 26.35 | tetradecanal | 0.65 |
| 31 | 27.43 | myristic acid | 3.25 |
| 32 | 27.77 | asaron | 7.80 |
| 33 | 28.84 | 5,10-pentadecadienol | 0.47 |
| 34 | 28.95 | phytol | 0.95 |
| 35 | 29.37 | scytalon | 3.30 |
| 36 | 30.43 | hyperforin | 2.55 |
| 37 | 30.61 | 2-heptadecenal | 0.45 |
| 38 | 31.47 | Palmitic acid | 3.05 |
| 39 | 31.68 | Margaric acid | 25.88 |
| 40 | 34.08 | 4,6,9-nonadecatriene | 1.79 |
| 41 | 34.13 | 3-méthyl,2- butene2-one | 0.65 |
| 42 | 34.67 | myristicalcool | 18.42 |

*Antimicrobial properties*

The antimicrobial properties were examined by the disk-diffusion method (Bauer, *et al*., 1966). The bacterial cell suspension was prepared for 24h culture and adjusted to an inoculation of 1 × 106 CFU/ml. Sterile nutrient agar (Immunpräparate, Berlin, D, 26g agar/L distilled water) was inoculated with bacterial cells (100 µl f bacterial cell suspension in 25 ml medium) and poured into dishes to give a solid plate. Yeasts and fungi (1 × 107 CFU/ml) were inoculated into sterile Mueller-Hinton-agar (Becton Dickinson, Heidelberg) according to DIN E 589440-3 for the agar disc-diffusion assay (3, 7). 20µ l of test material (250g/ 500 ml) dissolved in the same solvent like for the extraction, were applied on sterile paper discs (6 mm) diameter. Ampicillin, gentamicin and nystanin, were used as positive control, and the solvents (Water and Methanol-water 50/50 (v/v)) were used as negative control. The solvent were evaporated in a stream of air and the discs were deposited on the surface of inoculated agar plates. Plates were kept in a refrigerator for 1 h to permit good pre-diffusion of substances into the agar. The plates with bacteria were incubated for 24 h at 34°C, the plates with yeast for 36°C and the plates with fungi for 72h at 30°C. Inhibition zone diameters around discs (diameter of inhibition zone plus diameter of the disc) were measured. An average zone of inhibition was

calculated for the three replicates. Minimal inhibitory concentration (MICs) was determined by the agar diffusion technique as described by Muanda *et al*. (4). The highest concentration of the extract tested was 1.5 mg/ml. The MIC corresponds to the lowest concentration of the tested extracts able to inhibit any visible microbial growth. Several concentrations of the extracts were prepared (0.25, 0.20, 0.15, 0.10, 0.05, 0.005 ml/ ml). The solutions were agitated vigorously. 20 µl of each concentration was transferred into the disc. Then the discs were transferred in the Petri dishes containing the testing microorganisms. The plates were incubated at 30°C (72h), 36°C (48h) and 37°C (24h) for the fungal, the yeast and the bacterial respectively. After incubation the numbers of colonies in each plate were counted. Each assay replicated tree times.

The following bacterial strains were employed in the screening:

*Staphylococcus aureus* (ATCC29213), Bacillus subtilis (ATCC6059), *Escherichia coli* (ATCC25922), Pseudomonas aeruginos (ATCC6059) and fungi: *Aspergillus niger* (135550/99), *Candida albicans* (ATCC90028). The antimicrobial properties were examined by the disk-diffusion method (7). The bacterial cell suspension was prepared from 24h culture and adjusted to an inoculation of 1 × 106 CFU/ml. Sterile nutrient agar (Immunpräparate, Berlin, D, 26g agar/L distilled water) was inoculated with bacterial cells (100 µl f bacterial cell suspension in 25 ml medium) and poured into dishes to give a solid plate.

Yeasts and fungi (1 × 107 CFU/ml) were inoculated into sterile Mueller-Hinton-agar (Becton Dickinson, Heidelberg) according to DIN E 589440-3 for the agar disc-diffusion assay (8). 20 µl of test material (250g/ 500 ml) dissolved in the same solvents like for the extraction, were applied on sterile paper discs (6 mm) diameter. Ampicillin, gentamicin and nystanin, were used as positive control, and the solvents (Water and Methanol-water 50/50 (v/v)) were used as negative control. The solvent were evaporated in a stream of air and the discs were deposited on the surface of inoculated agar plates. Plates were kept in a refrigerator for 1 h to permit good pre-diffusion of substances into the agar. The plates with bacteria were incubated for 24 h at 37°C, the plates with yeast for 48h at 36°C and the plates with fungi for 72h at 30°C. Inhibition zone diameters around discs (diameter of inhibition zone plus diameter of the disc) were measured. An average zone of inhibition was calculated for the three replicates. Minimal inhibitory concentration (MICs) was determined by the agar diffusion technique as described by Muanda *et al*., (3). The highest concentration of the extract tested was 1.5mg/ml. The MIC corresponds to the lowest concentration of the tested extracts able to inhibit any visible microbial growth. Several concentrations of the extracts were prepared (0.25, 0.20, 0.15, 0.10, 0.05, 0.005 ml/ ml). The solutions were shaken vigorously. 20 µl of each concentration was transferred into the disc. Then the discs were transferred in the Petri dishes containing the testing microorganisms. The plates were incubated at 30°C (72h), 36°C (48h) and 37°C (24h) for the fungal, the yeast and the bacterial respectively. After incubation the numbers of colonies in each plate were counted. Each assay replicated tree times. For the antimicrobial analysis, the results of antimicrobial test are shown in table 3 and figure 3 a, 3b.

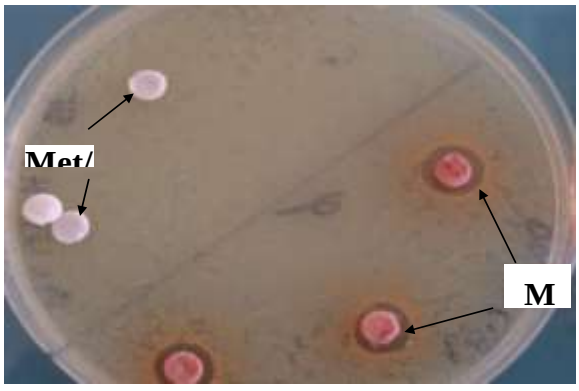


**Figure 3a**. *V. doniana*(Met/W) contrôle (-): MWe (50%) (*E.coli*)

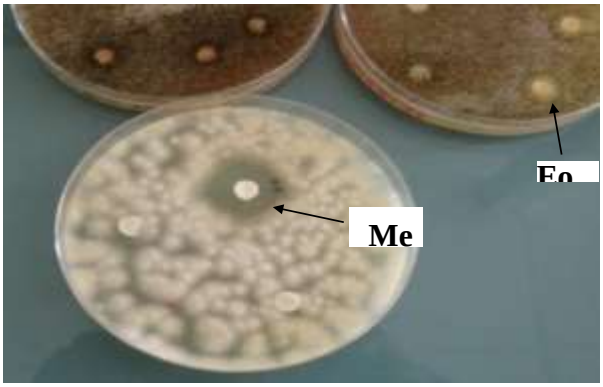

**Figure 3b**. *V. doniana*: MWe (50%) (C.a)

## Discussion

About 42 compounds are identified in the *V. doniana* essential oils. The results show that the major compounds are margaric acid (25, 88%), myristic acid (18.42%), asaron (7.80%), scytalon (3.30%), hyperforin (2.55 %), linanool (1.91%).

Most of the compounds identified play important roles in the aromas of essential oils, for example, carophyllene possess a wood spicy odourand has been used particularly for chewing-gum as well as in spice blends and flavour compositions (3-4, 9). But several authors showed that the relative composition of some compounds change significantly because of the use of dry leaves (3-4). Volatile aroma compounds are the most sensitive compounds in the process of food drying but the loss of volatiles in herbs and spices during the process depends mainly on drying conditions and the biological characteristics of plants. The antioxidant activity of essential oils was also affected (3-4, 10).

Quantitative and qualitative analysis of individuals phenolic compounds by RP-HPLC show that water is not the best extract solvent for phenolic compounds (6). Muanda *et al*. (6), reported that an anqueous alcohol is considered tobe the best solvent for extracting phenolic compounds from that materials. For the antioxidant properties, it is well known that plants containing various antioxidants such as tocopherols, polyphenols have antioxidant properties (6, 11). Phenolic compounds have received much attention as one of the principal antioxidants found in plants. Several authors have reported that some essential oils and organic solvent extracts from plants possess antioxidant activity (3-4, 12).

Phytochemical screening of *V. doniana* extract revealed the presence of phenolic compounds (6), the presence of these metabolites reveals its activity against pathogenic bacteria. According to Muanda (3-4) and Ebana (13), alkaloids inhibit pathogenic bacteria and tannins are important in herbal medicine in treating wounds and stopping beeding (6).

The extracts had varing degrees of antimicrobial activity against the test organisms. The MWE had the highest activity with the zone of inhibition between 9-20 mm as compared to that of WE/EO (table 3). This antioxidant activity may be due to the presence of terpene with antioxidant activity such as carophylene, linalool with strong antioxidant activity (3-4, 9). The results are in accordance with those found by Ali, *et al*. (1); Muanda *et al*. (3); Oyeleke *et al*. (14). The minimum inhibitory concentration (MIC) obtained reveals the effectiveness of the plant extract as chemotherapeutic agents. The organisms were inhibited at concentrations ≥ 1000 µg/ml (table 3). And the most susceptible organisms to the antimicrobial activity of *V. doniana* were *E. coli*, *Staphyloccocus aureus* and *Candida albicans*. These properties are correlated to the antioxidant properties.

In the present study, phenolic compounds and microbiological activity of the extracts of *V. doniana* leaves have been examined. The results indicated that from all the phenolic compounds identified in the extracts, rutin (11943 ug/ml) was the most abundant. On essential oils extract, the results demonstrate that the major compound is margaric acid (25, 88%). The antimicrobial activities showed that MWE extract was more effective against pathogens.

Weakness and strengths

In conclusion, the results of the present study can support the view that *V. doniana* leaves can be a potential source of natural antioxidant and microbial properties, which justifies its use in

folk medicine, it may be recommended for biotechnologies uses.

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**References**

1. Ali M., Aminu F., Ibrahim IS. In-vitro assessment of antibacterial activity and phytochemical screening of *Vitex doniana* on clinical isolate of salmonella typhi. International, *Journal of Advanced Academic Research* 2017; **3**: 2488-9849.
2. Siddique AB, Mizanur R., Amzad H. Chemical composition of essential oil by different extraction methods and fatty acid analysis of leave of Stevia Rebaudia Bertoni, *Arabian Journal of Chemistry* 2016; **9**: S1185-S1189.
3. Muanda F N., Soulimani R., Diop B., Dicko A. Study on chemical composition and biological activities of essential oil and extracts from Stevia rebaudiani leaves, LWT - *Food Science and Technology*, 2011 ; **3**: 1-8.
4. Muanda FN., Bouayed J., Djilani A., Yao CY, Soulimani R., Dicko A. Chemical Composition and, Cellular Evaluation of the Antioxidant Activity of Desmodium adscendens Leaves. Evidence-based Complementary and Alternative Medicine (eCAM), 2010a: 1-9.
5. Muanda FN., Dicko A., Soulimani R. Chemical composition and biological activities of Ficus capensis leaves extracts. *Journal of Natural Products (J.N.P)*, 2010; **3**:147-160.
6. Muanda FN., Koné D., Dicko A., Soulimani R., Younos C. Phytochemical Composition and Antioxidant Capacity of Three Malian Medicinal Plant Parts. Evidence-based Complementary and Alternative Medicine (eCAM), 2009; doi: 10.1093/ nep 109 1 - 8.
7. Bauer AW., Kirby WM M., Sheriss JC, Turck M. Antibiotic susceptibility testing by a standardized single disc method, *Am J Clin Pathol.* 1966; **45**: 493 - 496.
8. Al-Fatimi, M., Wurster, M., Schröder, G., Lindequist, U. Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. *J Ethnopharmacol* 2007; **111**: 657-666.
9. El-Massy KF, El-Ghorab AH, Shaaban HA, Shibamoto T. Chemical compositions and antioxidant/ antimicrobial activities of various samples prepared from Schinusterebinthifolius cultivated in Egypt. *J Agric FoodChem* 2009; **57**: 5265-5270.
10. Omidbaigi, R., Selidkom, F., Kazemi, F. Influence of drying methods on essential oil content and composition of Roman chamomile. *Fav Fragr J* 2004; **19**: 196-198.
11. Graddmann J. Terpenoids as plant antioxidants. *Vitam Horm* 2005; **72**: 505 - 535.
12. El-ghorab A, El- Massy KF, Shibamoto T. Chemical composition of the volatile extract and antioxidant activities of the volatile and non-volatile extracts of Egyptian corn silk (ZeamaysL.). *J Agric Food Chem* 2007; **55**: 9124 – 9127.
13. Ebana RUB., Madunagu BE, Ekpe ED, Otung IN. Microbiological exploitation of cardiac glycosides and alkaloids from Garcinia kola, Borreriaocymoides, Kola nitida and Citrus aurantifoli. *J Appl Biotechnol* 1991; **71**: 398 - 401.
14. Oyeleke SB, Dauda BEN., Boye OA., Antibacterial activity of Ficuscapensis. *Afr. J. Biotechnol* 2008; **7**: 1414 - 1417.

**Table 3. Antimicrobial analysis** (*V. doniana* extracts)

| | L. extracts | | | CN (-) | | CN (+) | | |
|---|---|---|---|---|---|---|---|---|
| | E.o | We | Mwe | W | Met | Ap | Gt | Ny |
| *E.c.* | 9.5 ± 0.2 | 12±0.1 | 20±0.3 | 0 | 0 | 58±0.3 | 55±0.5 | n.a |
| *C.a.* | 14 ±0.2 | 10 ±0.3 | 16±0.2 | 0 | 0 | n.a | n.a | 53± 0.4 |
| *A.n.* | 19±0.4 | n.a | 15±0.1 | 0 | 0 | n.a | n.a | 47± 0.3 |
| *P.a.* | n a | 13±0.3 | 14±0.2 | 0 | 0 | 45±0.2 | 45± 0.4 | na |
| *B.s.* | 12±0.1 | 12±0.1 | 9±0.4 | 0 | 0 | 55±0.3 | 45±0.3 | na |
| *S.a.* | 12±0.3 | 9 ±0.2 | 19 ±0.1 | 0 | 0 | 56±0.4 | 44± 0.2 | na |

Organisms Zone of inhibition (in mm)

Value are mean ± SD of three determinations

E.c.: *Escherichia coli*; C.a: *Candida albicans;* A.n : *Aspergillus niger*; B.s.: *Bacillus subtillus* S.a.; *Staphyloccocus aureus;* P.a.: *Pseudomonas aeruginosa*, Ny (+) Nystanin positif control ; Gt (+) gentamicin (positif control) ; N.a : not actif, Ap ( +): Ampicillin (+) positif control, We (water) (-) Negatif control; Me (methanol)(-)(Negatif control), Mwe ( methanol water extract) ; E.o (Essential oil extract); CN (control)