Comparative Studies of the Phytochemical and Antioxidant activities of the leaves, stems, roots, and seeds extracts of *Mitracarpus scaber* Zucc (Rubiaceae)

Bea Gouanda Thibaut 1,3, Coulibaly Wacothon Karime 2,3, Benie Anoublé 1, Kablan Ahmont Landry Claude 2, James Tembei Titah 4, N’Guessan Hugues Alain 1, Kabran Aka Faustin 3, Konan Dibi Jacques 3, and Attioua Koffi Barthélemy 3

1 Laboratoire de Chimie Bio-Organique et de Substances Naturelles, Université Nangui Abrogoua, Abidjan, Côte d’Ivoire

2 UPR de chimie organique, Département de Mathématiques, Physique et Chimie, UFR des Sciences Biologiques, Université Peleforo Gon Coulibaly, Korhogo, Côte d’Ivoire

3 Laboratoire de Constitution et Réaction de la Matière, UFR Sciences des Structures de la Matière et Technologie, Université Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d’Ivoire

4 Tabor College, Department of Chemistry-Science & Mathematics, Hillsboro, KS, USA

**Abstract:** Extracts of the leaves, stem, roots, and seeds of *Mitracarpus scaber* plants have been thoroughly investigated in an attempt to determine their phytochemical and antioxidant activities. Phytochemical screening carried out by thin-layer chromatography (TLC) revealed the presence of several secondary metabolites in all the selective extracts of the plant. The levels of phenolic compounds showed that the leaves of *M. scaber* are the richest in polyphenols with an average value of 488.291 ± 1.205 μg GAE/g (microgram of gallic acid equivalent per gram of the dry matter of the powdered plant). The total flavonoid assay revealed a high content in the leaves (1.624 ± 0.032 %). Also, studies of the antioxidant activities by DPPH* (2,2-diphenyl-1-picrylhydrazyl) method showed a significant effect compared to vitamin C (96.62 %). The percentages of inhibition are respectively 89.82 % and 82.3 % for ethyl acetate extracts of the leaves and seeds. Furthermore, the inhibitory percentages of n-butanol in the leaves and seeds are 90.57 % and 83.67 %, respectively. Calculated IC50 showed that the n-butanol fraction of the seeds exhibited the highest activity with an IC50 of 0.293 mg/mL less than ascorbic acid with an IC50 of 0.387 mg/mL.

**Keywords:** Rubiaceae; *Mitracarpus*; Phytochemical; Polyphenols; Antioxidant.

1. Introduction

The use of plant extracts in the treatment of various diseases in humans has existed for decades, and the research is ongoing. Different parts of plants such as the leaves, stem, barks, roots, etc. are being used to prevent, alleviate symptoms or revert abnormalities in humans. Although these traditional therapies have been ongoing for a long time, the dangers, including cases of burns and intoxications have been observed from extracts containing very toxic and harmful substances. Nevertheless, these natural and traditional practices or therapies are in rising demands due to the ever-increasing and challenging health crisis in the world, especially in developing countries. In order to combat the numerous phenomena of bacterial resistance in humans and animals, new therapeutic agents must be produced from plants. They are at the center of multiple studies for their use in the treatment of several pathologies. 1,2 *M. scaber* Zucc (Rubiaceae) is being used in traditional medicine to treat certain skin diseases such as eczema and ringworm. 3 Despite its use in traditional medicine, very few scientific studies have been conducted on this species, hence the importance of new research on the various organs (roots, stems, leaves, and seeds) of this plant. Therefore, the objective of this study is to determine the phytochemical and antioxidant properties of *M. scaber* in the extracts of the leaves, stem, roots, and seeds. The present work is done to contribute to the valorization of medicinal plants used in the Ivory Coast.

2. Experimental

2.1. Materials and methods

2.1.1. Materials
2.1.1.1. Plant material
The leaves, stems, roots, and seeds of *M. Scaber* were collected in July 2017 at Nangui Abrogoua University (Ivory Coast). They were identified by Dr. DJAH Malan Jean-Francois, Professor-researcher, in Botany at the said University. These parts were washed twice with tap water, were dried for one month and three days. They were then pulverized through an artisanal mill.

2.1.1.2. Technical material
Technical equipment used: a precision balance (Denver, S-234 series, Max 230g), a Buchner, a UV lamp (254 and 366 nm), a spectrophotometer (AL8000Aquatic series), a rotary evaporator of HEIDOLPH type and an electric dryer. The deposits on thin-layer chromatography (TLC) were carried out on chromatographic plates bought in the starts (silica gel 60 F254, aluminum, 20×20 cm, Merck).

2.1.2. Experimentation
2.1.2.1. Extraction of secondary metabolites
2.1.2.1.1. Maceration
Each powdered plant material (5.00 g) was macerated in 50.00 mL of methanol (80 %) with constant stirring for 24 hours. The extract was obtained through suction filtration and stored between 4-5°C in a refrigerator. This operation was repeated twice, keeping the same pomace, but with a fresh solvent. Hydromethanol macerates were combined in different flasks and then concentrated under reduced pressure at 50°C with a rotary evaporator to give four crude hydromethanol extracts (leaves, stem, roots, and seeds) from *M. scaber*. After concentration, the resulting hydromethanol crude extracts were dried in an oven and then weighed using a precision balance (Denver, S-234 series, Max 230g) for the calculation of the yield. These extracts were subsequently used to prepare selective extracts and carry out the different assays.

2.1.2.2. Preparation of selective extracts
Extracts from the leaves, stems, roots, and seeds were respectively treated with the following increasing polarity solvents: hexane, chloroform, ethyl acetate, and n-butanol. The crude hydromethanol extract (20.00 mL) was successively extracted with 3x10 mL of hexane, chloroform, ethyl acetate, and n-butanol. The different fractions obtained were then stored in a refrigerator (4–5°C) for subsequent use in phytochemical screening and antioxidation activity.

2.1.2.3. Phytochemical screening of selective extracts
The different extracts obtained from hexane, chloroform, ethyl acetate, and n-butanol were identified and analyzed using Thin Layer Chromatography (TLC) 

2.1.2.4. Determination of total polyphenols
Total polyphenol content was determined using the colorimetric method proposed by Folin-Ciocalteu and used by other authors. $1.00 \text{ mL}$ solution of each extract was diluted to 1/20 with distilled water. 1.50 mL of Na$_2$CO$_3$ (17 %, w / v) and 0.50 mL of Folin-Ciocalteu reagent (0.5 N) was added to the resulting solution. The absorbances were determined at 720 nm using a spectrophotometer. The total phenolic content was also determined using a standard made up of different concentrations of gallic acid ($y = 0.0083x + 0.0147$).

2.1.2.5. Determination of total flavonoids
Total flavonoid assay was performed using the method proposed by Hariri et al., (1991) and used by Kabran. In this method, 2 mL of each hydromethanol extract was diluted to 1/20 with distilled water and mixed with 100 μL of Neu reagent. The absorbance was read at 404 nm, and the result compared to that of quercetol taken as standard (0.05 mg/mL), diluted under the same conditions, and treated with the same amount of reagent. The percentage of total flavonoids was calculated in quercetol equivalent according to the following formula below proposed by Hariri et al., (1991).

$$F(\%) = \frac{Aq \times 100 \times d}{A_{ext}}$$

Where; $F(\%) =$ percentage of total flavonoids; $A_{ext} =$ Absorbance of the extract; $Aq =$ Absorbance of quercetol; $d =$ dilution; $C_{ext} =$ Concentration of extract (mg/mL).

2.1.2.6. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity
The antioxidant activity of the extracts was carried out using the spectrophotometric method proposed by Popovic et al. and repeated by N’Gaman, (2013). The (2,2-diphenyl-1-picrylhydrazyl) DPPH’ was dissolved in absolute ethanol to obtain a concentration of 0.02 mg/mL. Similarly, solutions of the extract were prepared in absolute ethanol with varying concentrations (0.25 mg/mL, 0.50 mg/mL, 1.00 mg/mL, 1.50 mg/mL, 2.00 mg/mL and 3.00 mg/mL). In a dry and sterilized test tube, 0.50 mL of the different extract concentrations was mixed with 1.50 mL of the solution of DPPH at 30 minutes interval. The resulting mixture was thoroughly mixed and placed in the dark for 15 minutes. The absorbance of the mixture was measured using a spectrophotometer 517 nm (maximum wavelength of DPPH). The absorbance was measured at 3 minutes’ interval for 15 minutes. This process was repeated for the different extract solutions. Ascorbic acid (vitamin C) was used as a reference for positive control. The scavenging effect was calculated as:

$$\% I = (A_0 - A_i) \times 100/A_0$$
Where $A_0$ = absorbance of the control; $A_1$ = absorbance of the extract.

A standardized graph of the extract concentration versus the scavenging activity was plotted to extrapolate the concentration efficiency of the plant extract at 50 % reduction of DPPH (IC$_{50}$) $^6$.

3. Results and discussion

3.1. Phytochemical Screening

3.1.1. Hexane extract

In this method, family-specific reagents were used to determine large families of secondary metabolites. Steroids were determined using the Godin's reagent. This was seen as purple or brown spots in the visible region $^8$. According to Lagnika (2005) $^{15}$ and Kabran (2014) $^3$, steroids are identified using the Libermann-Burchard reagent as brown and green spots in the visible region, and yellow and yellow-green spots in the UV region at 366 nm. Similarly, terpenes are identified as blue and purple spots in the visible region. Reports from Ogunleye et al. also confirmed these results. (2019) $^{15}$ with ethanol extract of $M$. scaber leaves.

In addition, solutions of methanol containing 5 % KOH were used to determine coumarins. It was observed that only extracts from the roots confirm the presence of coumarins seen as a blue spot (Rf = 0.53) in the UV region at 366 nm, as approved by Ladiguina et al. $^{16}$. The other plant extracts (seeds, stems, and leaves) do not show the presence of coumarins, but they contain sterols and terpenes, as shown in Table 1.

**Table 1. Phytochemical constituents of hexane extract of leaves, stems, roots, and seeds of $M$. scaber.**

| Secondary metabolites | leaves | stems | roots | seeds |
|-----------------------|--------|-------|-------|-------|
| Sterols and terpenes  | +      | +     | +     | +     |
| Coumarins             | -      | -     | +     | -     |

(+) = presence; (-) = absence

3.1.2. Chloroform extracts

Specific reagents were used to identify the different large families of secondary metabolites.

Dragendorff reagent was used to determine the presence of alkaloids in the extracts. After treating the different extracts of chloroform with Dragendorff reagent, no spot was observed on the chromatographic plate, indicating the absence of alkaloids. In addition, the Neu reagents, in combination with AlCl$_3$, were used to determine the presence of flavonoids in the different plant extracts.

Our results indicate the presence of flavonoids in all the plant extracts (leaves, stem, roots, and seeds) observed as blue spots on the chromatographic plates with Rf values of 0.71 and 0.63. These results are in agreement with previous reports by Ogunleye et al. $^{15}$. This also confirms the presence of flavones and isoflavones, as previously reported $^{11,14,17,21}$. Similarly, the different plant extracts (leaves, stem, roots, and seeds) obtained from methanol in 5 % KOH reveal the presence of coumarins with Rf value of 0.76. The results are summarized in Table 2.

**Table 2. Phytochemical constituents of chloroform extracts of leaves, stems, roots, and seeds of $M$. scaber.**

| Secondary metabolites | leaves | stems | roots | seeds |
|-----------------------|--------|-------|-------|-------|
| Coumarins             | +      | +     | +     | +     |
| Flavonoids            | +      | +     | +     | +     |
| Alkaloids             | -      | -     | -     | -     |

(+) = presence; (-) = absence

3.1.3. Ethyl acetate extracts

To determine organic compounds present in the extract from ethyl acetate, 3:0.2 (v/v) AcOEt/AcOH solvent mixture was used as the extracting solvent. The results show the presence of flavonoids in all the extracts and no alkaloids even after using Dragendorff reagent, which is specific to alkaloids (Table 3) $^{14}$. This result is contrary to previous reports from Ogunleye et al. $^{15}$.

**Table 3. Phytochemical constituents of ethyl acetate extract of leaves, stem, roots, and seeds of $M$. scaber.**

| Secondary metabolites | leaves | stems | roots | seeds |
|-----------------------|--------|-------|-------|-------|
| Flavonoids            | +      | +     | +     | +     |
| Alkaloids             | -      | -     | -     | -     |

(+) = presence; (-) = absence
3.1.4. n-butanol extracts
The EtOAc/EtOH/ACOH/H2O solvent system in the ratio 4: 0.6: 0.5 v / v / v / v was used to analyze the extracts prepared from n-butanol. After carrying out the phytochemical screening on the extracts, results reveal the presence of tannins, polyphenols, flavonoids, and coumarins in all the extracts (Table 4). Summarily, TLC analysis on the extracts prepared from hexane, chloroform, ethyl acetate, and n-butanol, reveal the presence of sterols, terpenes, coumarins, flavonoids, tannins, and polyphenols.

Table 4. Phytochemical constituents of n-butanol extracts of leaves, stem, roots, and seeds of M. scaber.

| Secondary metabolites       | leaves | stems | roots | seeds |
|----------------------------|--------|-------|-------|-------|
| Coumarins                  | +      | +     | +     | +     |
| Flavonoids                 | +      | +     | +     | +     |
| Tannins and polyphenols    | +      | +     | +     | +     |

(+) = presence; (-) = absence

3.2. Total polyphenol content
Results of the total polyphenol content in the plant extracts using hydromethanol as extracting solvent are presented in Figure 1.

From the results, the total polyphenol content in the crude extracts of M. scaber indicated that all the extracts (leaves, roots, seed, and stem) contain polyphenols ranging from 174.059 to 488.291 ± 1.205 μg GAE/g of the dry matter. The leave extracts of M. Scaber contain the highest amount of polyphenols (488.291 ± 1.205 μg GAE/g), followed by extracts from the seeds (344.277 ± 0.696 μg GAE/g) and then the stems (237.538 ± 1.205 μg GAE/g), with the roots containing the least amounts of polyphenols (174.059 ± 1.840 μg GAE/g). These results are significant and consistent with a previous study carried out with Butanol-HCl 17,22,23. Besides, these reports have confirmed that polyphenols possess numerous pharmacological and biological properties, including their use as antioxidants, antibacterial, anti-inflammatory agents, etc. Derivatives of polyphenols such as tannins and flavonoids have been used as antioxidants to fight against certain body disorders caused by oxidative stress 24-26.

![Figure 1. Total polyphenols content in different plant extracts of M. scaber](image)

F= leaves, T = stem, R= root, G= seeds

3.3. Total flavonoid content
Total flavonoid contents in the extracts of leaves, stems, roots, and seeds of M. scaber with methanol used as the extracting solvent are presented in Figure 2. Similarly, flavonoids are unevenly distributed in M. scaber plant with percentages ranging from 0.6166 ± 0.0001 % - 1.6236 ± 0.0317 %). The highest flavonoids content are found in the leaves (1.6236 ± 0.0317 %), followed by the seeds (1.3780 ± 0.0248 %), the stem (0.7812 ± 0.0135 %), with the roots having the least amounts of flavonoids (0.6166 ± 0.0001 %). These results are in agreement with earlier results from Kambo et al., (1999) 27.

The increased flavonoid content in the leaves and seeds can be attributed to their exposure to sunlight compared to other parts of the plant 10,11.
3.4. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of n-butanol extracts

Figure 3 represents the inhibition of the various extracts with n-butanol in relation to the behavior of DPPH*. These extracts were incubated at 3 minutes interval for 15 minutes. The different methods used are the FRAP method, the ORAC, and the radical DPPH* method. The preference for the radical scavenging DPPH* method in the determination of antioxidant activity in compounds or extracts is due to its simplicity and ease of use, its speed, and the formation of a stable radical. The antioxidant properties of the extracts were determined with reference to ascorbic acid (vitamin C), which is considered a natural oxidant. The results revealed that all the extracts exhibited anti-radical effects as a function of time. An increase or a decrease in the percentage of inhibition was observed every 3 minutes. Similarly, the rate of inhibition also varies as a function of time. This trend was also observed in ascorbic acid (vitamin C), which is the reference molecule with a radical-activity value of 96.617%. In the n-butanol extracts, the radical scavenging activity was 90.57 % and 83.67 % in the leaves and seeds, respectively. These results are in agreement with those obtained by TLC. From Figure 3, it is seen that the antioxidant activity in the extracts is similar to those observed in vitamin C. In general, results of anti-radical activity using the spectrophotometer were in agreement with the assays of total polyphenols and total flavonoids. In addition, our study revealed the behavior of DPPH on our hydromethanol extracts as a function of time.
3.4.1. Determination of inhibition concentrations (IC$_{50}$)

The inhibition concentration (IC$_{50}$) is defined as the concentration of a compound at 50 % efficacy in inhibiting a biological function. The effectiveness of a compound in inhibiting a biological function is inversely related to its IC$_{50}$. The lower the IC$_{50}$ of a compound, the higher its ability to inhibit a biological function and vice versa.

The antioxidant activity of the extracts was determined from the IC$_{50}$ in comparison to the standard (vitamin C). The efficient concentration of the plant extract to which 50 % of the DPPH has been reduced (IC$_{50}$) was determined graphically from the graph of the standardized extract concentration versus the scavenging activity.

The results obtained showed that the seed extracts prepared from n-butanol fraction exhibit the most crucial activity with an IC$_{50}$ lower than that of ascorbic acid. This implies that flavonoids, which are abundant in the seeds, have the highest antioxidant activity.

Table 5. IC$_{50}$ values for n-butanol, ethyl acetate extracts, and vitamin C.

| Extracts (mg/mL) | Time   | 3 min | 6 min | 9 min | 12 min | 15 min |
|------------------|--------|-------|-------|-------|--------|--------|
| n-butanol leaves |        | 1.21  | 1.23  | 1.79  | 1.76   | 1.85   |
| n-butanol stem   |        | 1.39  | 1.37  | 1.33  | 1.35   | 1.29   |
| n-butanol roots  |        | 0.79  | 0.73  | 0.76  | 0.72   | 0.99   |
| n-butanol seeds  |        | 0.27  | 0.25  | 0.24  | 0.23   | 0.29   |
| AcOEt leaves     |        | 1.59  | 1.77  | 1.73  | 1.81   | 1.45   |
| AcOEt stem       |        | 0.95  | 0.85  | 0.59  | 0.81   | 0.80   |
| AcOEt roots      |        | 0.79  | 0.76  | 0.72  | 0.73   | 0.99   |
| AcOEt seeds      |        | 0.49  | 0.48  | 0.47  | 0.48   | 0.39   |
| Vit. C           |        | 0.35  | 0.36  | 0.37  | 0.36   | 0.39   |

4. Conclusion

The present study is, therefore, an essential contribution to the chemistry of *Mitracarpus scaber* Zucc. (Rubiaceae), to understand the effects of the use of this plant in the treatment of certain diseases in the Ivory Coast.

The phytochemical and antioxidant activities of different parts of the plant were successfully analyzed, *M. scaber*. Hexane, chloroform, ethyl
acetate and n-butanol extracts of the plant leaves, stems, roots, and seeds contain flavonoids, coumarins, tannins, sterols, polyphenols, and terpenes. Our results show high amounts of polyphenols and flavonoids in the leaves and seeds of the plant. The total flavonoid assay revealed a high content in the leaves (1.624 ± 0.032 %). This can be attributed to their frequent exposure to sunlight compared to the other parts of the plant. Our comparative studies indicate an uneven distribution of secondary metabolites in the different parts of the plant—with secondary metabolites being absent in some parts. Besides, the studies of the antioxidant activities by DPPH* (2,2-diphenyl-1-picrylhydrazyl) method showed a significant effect compared to vitamin C (96.62 %). The percentages of inhibition are respectively 89.82 % and 82.3 % for ethyl acetate and n-butanol extracts of the leaves and seeds. The DPPH* spectrophotometric tests revealed that the leaves and seeds of the plant with higher contents in polyphenols and flavonoids also have good antioxidant activity.

In several serious diseases, notably, those linked to aging, oxidative stress is the primary triggering factor. Since the n-butanol extract has shown good antioxidant activity by the DPPH method, it would be essential for us to confirm the antioxidant activity by performing other methods such as the Frap and Abts methods. The use of this plant could help us in the prevention of certain diseases linked to oxidative stress. Subsequently, we will perform biological antifungal and antibacterial tests to confirm the traditional use of the plant.

Acknowledgments

The authors are grateful to Prof. Attiouna Koffi Barthelemy of Laboratoire de Chimie Organique et de Substances Naturelles, UFR Sciences des Structures de la Matière et Technologie, Université Félix Houphouët-Boigny, Abidjan, Côte d’Ivoire, for phytochemical studies.

References

1- K. H. Baser, G. Tümen, N. Tabanca, F. Demirci, Composition and antibacterial activity of the essential oils from *Satureja wiedemanniana* (Lallem.). *Velen. Z., Natur for sch.*, 2001, 56, 731-738.
2- J.K. Chalchat, L.P. Carry, C. Menut, G. Lamaty, R. Malhuret, J. Chopineau, Correlation between chemical composition and antimicrobial activity. VI. Activity of some African essential oils, *J. Essent. Oil Res.*, 1997, 9, 67-75.
3- E.J. Adjahounou, La médecine traditionnelle au Bénin : recensement des savoir- faire traditionnels, *Méd. Pharm. Afr.*, 2001, 15, 103-111.
4- T.O. Ekpendu, P.A. Akah, A.A. Adesomoju, J.I. Okogun, Antiinflammatory and antimicrobial activities of *Mitracarpus scaber* extracts, *International Journal of Pharmacognosy*, 1994, 32, 991-2196.
5- Y.A. Békro, J. Y.-A. Mamyrbekova-Békro, B.B. Boua, F. Tra Bi, E. Ehlié, Etude ethnotobanique screening phytochimique de *Caesalpinia benthamiana* (Baill.) Herend. et Zarucchi (*Caesalpiniaeae*), *Sciences et Nature*, 2007, 4, 217-225.
6- J. Y.-A. Mamyrbéko-Békro, M. Konan, Y.A. Békro, M. Djie Bi, T. Zomi Bi, V. Mambo, B.B. Boua, Phytoconstituents of the extracts of four medicinal plants of Côte d’Ivoire and assessment of their potential antioxidant by thin-layer chromatography, *European Journal of Scientific Research*, 2008, 24, 219-228.
7- K.C.C. N’gaman, Y.-A. Békro, J. Mamyrbékova-Békro, A. Bénie, B.S. Gooré, Sur la composition en métabolites secondaires et l’activité anti-oxydante d’extraits bruts de *Gmelina arborea* Roxb. (Verbanaceae) de Côte d’Ivoire, Afrique de l’Ouest : Analyse par Chromatographie sur Couche Mince, *European Journal of Scientific Research*, 2009, 36, 161-171.
8- G. R. M. Kabran, N. C. Ambeu, J. A. Mamyrbêko-Békro, Y.-A. Békro, Total Phenols and Flavonoids in Organic Extracts of Ten Plants used in Traditional Therapy of Breast Cancer in Côte d’Ivoire, *European Journal of Scientific Research*, 2012, 68, 182-190.
9- A. B. KADJA, J. A. Mamyrbekova-Békro, A. Benie, *Erythrophleum africanum Afsel*. (Caesalpiniaceae), an African toothpick: Phytochemical screening, total flavonoid content and antioxidant activity, *Journal of Medicinal Plants Research*, 2011, 5(27), 6273-6277.
10- A-H. O. N’Guessan, C. E. Dago, J. A. Mamyrbékova-Békro, Y.-A. Békro, Teneurs en composés phénoliques de 10 plantes médicinales employées dans la tradithérapie de l’hypertension artérielle, une pathologie émergente en Côte d’Ivoire, *Revue de Génie Industriel*, 2011, 6, 55-61.
11- C. C. N’gaman, G. R. M. Kabran, B. A. Kadja, J. A. Mamyrbêko-Békro, J. L. Pirat, M. Lecouvey, O. Sainte-Cathérine, N. Sommerer, A. Verbaere, E. Meudec, Y. A. Békro, ULPC-MS/MS phenolic quantification and in vitro anticancer potential of *Gmelina arborea* Roxb. (Verbanaceae). Der Chemica Sinica, 2014, 5(6), 13-17.
12- E. B. Hariri, G. Sallé, C. Andary, Involvement of flavonoids in the resistance of two poplar cultivars to mistletoe (*Viscum album* L.), *Protoplasma*, 1991, 16, 20-26.
13- C. Popovicu, I. Saykova, B. Tylkowski, Evaluation de l’activité antioxydante des composés phénoliques par la réactivité avec le
radical libre DPPH, *Revue de génie industriel*, 2009, 4, 25-39.

14- B. Wenigerat, L. Lagnika, C. Vonhron-Sénécheau, T. Adjobimy, J. Glenou, M. Moudachirou, R. Brun, R. Anton, A. Sanni, Evaluation of ethnobotanically selected Benin medicinal plants for their in vitro antiplasmodial activity, *Journal of Ethnopharmacology*, 2004, 90, 279-284.

15- A.O. Ogunnelaye, O.D. Florence, M.T. Hassan, Phytochemical and antimicrobial activity of the ethanol leaf extract of *Mitracarpus scaber*, *FUDMA Journal of Sciences (FJS)*, 2019, 3, 1-2.

16- E.Y. Ladiguina, L.N. Safronitch, V.E. Otriachenkova, I.A. Balandina, N.I. Grinkevitich, Analyse chimique des plantes médicinales. Edition Moskva, VischayaChkola, 1983, 347 p. (Translated from Russian).

17- K. M. Konan, J. A. Manyrbéka-Béko, Y.A Béko, Quantification of total phenols and flavonoids of *Desmodium adscendens* (Sw.) DC. (Papilionaceae) and projection of their antioxidant capacity, *J. Appl. Biosci*. 2012, 49, 3355–3362.

18- N. Ali, M. Moudachirou, J.A. Akakpo, J. Quetin-Leclercq. Treatment of bovine dermatophilosis with *Cinnamala*, *Lantana camara* et *Mitracarpus scaber*, *J. Ethnopharmacol*. 2003, 86, 167-171.

19- Mohammedi Z., F. Atik, Antioxidant Activity of Four Algerian Plants: *Cistus ladaniferus*, *Crataegus oxyacantha*, *Lavandula stoechas* et *Smyrnium olusatrum*, *Asian Journal of Chemistry*, 2011, 23(2), 709-712.

20- K.R. Markham, Techniques of flavonoid identification. Biological techniques Series. EditionTreherne J. E., Rubery P. H. *Academic press.*, 1982, 113p.

21- B.P. Georgivskii, N.F. Komissarenko, Dmitrou C.E. (1990). Les composés bioactifs des plantes médicinales, édition Naouka. 336 p (traduit du Russe).

22- B. Ouadja, K. Anani, B. Djori, Y.O. Amoyapoh, D.S. Karou, Evaluation of the phytochemical composition, antimicrobial and anti-radical activities of *Mitracarpus Scaber* (Rubiacaeae), *J. Med. Plants Res.*, 2018, 12(28), 493-499.

23- H. Falleh, R. Ksouri, C. AbdeIly, Activité antioxidante et contenu en polyphenols dans les différents organes de l’artichaut sauvage, *Cynaracardunculus, Revue des Régions Arides, numéro spécial SIPAM*, 2006, 26, 341-344.

24- O.M.P. Sharma, T.K. Bhat, (2009), DPPH antioxidant assay revisited. *Food Chemistry*, 113, 1202 p.

25- R. Amarowicz, Tannins: the new natural antioxidants? *Eur. J. Lipid. Sci. Technol.*, 2007, 109, 549–451.

26- H. Falleh, R. Ksouri, K. Chaieb, B. Karray, N. Trabelsi, M. Oulaiba, *et al.*, Phenolic composition of *Cynara cardunculus* L. organs, and their biological activities, *CR Biol.*, 2008, pp. 331-372.

27- a) K. E. Kporou, A. K. M. Kra, S. Ouattara, F. Guede-Guina, J. Djaman-Allico, Improvement by chromatographic dividing of anticandidosic activity of an hexanic extract *Mitracarpus scaber* on the in vitro growth of *Candida albicans* and *Candida tropicalis*, *Phytothérapie*, 2000, 8(5), 290-294.

b) N. A. Emmanuel, M. Moudachirou, A.J. Akakpo, J. Quetin-Leclercq, Activités antibactériennes in vitro de *Cassia alata*, *Lantana camara* et *Mitracarpus scaber* sur *Dermatophilus congolensis* isolé au Bénin, Revue Elev. Méd. Vet. Pays tropical, 2002, 55(3), 183-187.

28- I. Tagnaout, H. Zerkani, S. Amine, K. Fadili, N. Benhlima, A. Bouzoubaa, T. Zair. Phenolic composition and antioxidant potential of different solvent extracts of the endemic *Origanum elongatum* (Bonnet) *Emberger & Maire*, *Mediterranean Journal of Chemistry* 2020, 10(2), 146-154.

29- B. Bozin, N. Mimica-dukic, I. Smojlik, A. Goran, R. Igc, *Phenolics* as antioxidants in garlic (*Allium sativum* L., *Alliaceae, food chemistry*), 2008, 111, 925-929.

30- H. Dellaoiu, A. Berroucke, N. Halla, L. Boudaoud, M. Terras, Phytochemical study and evaluation of the antioxidant of *Myrtus communis* L. Fruit’s Methanol extract, *PhytoChem and BioSub Journal*, 2018, 12(2), 2170-1768.

31- I. Umadevi, M. Daniel, S. D. Sabnis, Chemotaxonomic studies on some members of Anardiaeaceae; In *Proceedings of the Indian Academy of Sciences, Plant sciences*, 1988, 98, 205–208.

32- A. Romani, P. Pinelli, N. Galardi, N. Mulinacci, M. Tattini, Identification and quantification of galloyl derivatives, flavonoid glycosides and anthocyanins in leaves of *Pistacia lentiscus* L., *Phytochemical Analysis*, 2002, 13, 79-86.

33- G. Chryssavgi, P. Vassiliki, M. Athanasios, T. Kibouri, K. Michael, Essential oil composition of *Pistacia lentiscus* L. and *Myrtus communis* L. Evaluation of antioxidant capacity of methanolic extracts, *Food Chemistry*, 2008, 107, 1120–1130.