Phytochemical Screening, Chemical Composition and Antioxidant Activity of Leaves and Bark Extracts from *Khaya senegalensis*

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Abstract: *Khaya senegalensis* belonging to family Meliaceae. This plant has been used as traditional medicine for several disease such as pain, inflammation, malaria, anemia, diabetic and gastrointestinal diseases. The objective of this study to carry out phytochemical screening, determination of chemical composition and determine antioxidant activity of plant extracts. Many of secondary metabolites were investigated in leaves extract include alkaloids, flavonoids, sterols and saponins while the bark extract includes sterols, triterpines, tannins and saponins. The antioxidant potential of ethanol extract of leaves and bark was determined on the basis of their scavenging activity of the stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical. Antioxidant activity of leaves extract was higher than bark with (42.69%) and (36.36%) respectively. The quantitative analysis of chemical composition was determined by Gas Chromatography–Mass Spectrometry (GC-MS). A number of bioactive compounds were present in the leaves and bark of *K.senegalensis*. The highest compound in both extracts represented the reducing sugar 4-O-Methyl mannose (66.47 and 14.73%) in bark and leaves respectively. Fatty acids and phenols were presented in high percentage in both extract include; n-Hexadecanoic acid, 9,12,15-Octadecatrienoic acid, Oleic Acid and Catechol.

Keywords: *Khaya senegalensis*, Leaves, Bark, Phytochemical Screening, GC-MS Analysis, Antioxidant Activity

1. Introduction

Traditional medicine is the sum total of knowledge, skills and practices based on the theories, believes and experiences related to different cultures that are meant to maintain health as well as to prevent, diagnose, improve or treat physical and mental illnesses. Medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing. Phytochemicals have two categories i.e., primary and secondary constituents. Primary constituents have chlorophyll, proteins, sugar and amino acids. Secondary constituents contain many compounds such as terpenoids and alkaloids. Phytochemicals are naturally occurring in the medicinal plants leaves, vegetables, fruits, bark and roots that have defense mechanism and protect from various diseases [1]. According to the World Health Organization Traditional herbal medicines are defined as naturally occurring, plant-derived substances with minimal or no industrial processing that have been used anciently to treat illness, within local or regional healing practices [2].

Herbal medicine is also called botanical medicine or phytomedicine. The extent to which it can be used is very broad, and the medicinal properties can be achieved by using a plant's seeds, berries, roots, leaves, bark, or flowers. Herbalism has a long tradition of use outside of conventional medicine. It is becoming more mainstream as improvements in analysis and quality control along with advances in clinical...
research show the value of herbal medicine in the treating and preventing disease [2]. *K. senegalensis* is a tall evergreen tree which grows in the Sahara savannah area from Senegal to Uganda. The extract from the bark of *K. senegalensis* is characterized by its bitter constituents, is named “calicedrin” in West Africa. It is used extensively as a bitter tonic for the treatment of a variety of pro-inflammatory disease. Commonly used in African traditional medicine for pain and inflammation [3]. The main chemical groups in *K. senegalensis* are fatty acids, carotenoids, coumarins, emodins, tannins, compounds reducers, anthracenosides, steroidal glycosides, flavonoids, carbohydrates, saponins, sterols and triterpenes, limonoids, anthocyanins, mono- and poly saccharides, mucilages and cardiac glycosides. Its bitter constituents, named “calicedrin” in West Africa, characterize the extract from the bark of *K. senegalensis*. It is used extensively as a bitter tonic for the treatment of a variety of pro-inflammatory disease [4].

### 2. Materials and Methods

#### 2.1. Preparation of Plant Materials

Dried leaves and bark of the plant materials of *K. senegalensis* were collected directly from the field at January 2018. The plant parts were thoroughly washed and air dried and ground to powder. One hundred gram of powdered material of leaves and bark were extracted with 96% ethanol for 72 hours at room temperature. Extracts were first filtered through Whatman No. 4 filter paper. After filtration, the extracts were vacuum concentrated.

#### 2.2. Methods

##### 2.2.1. Phytochemical Screening

Qualitative preliminary phytochemical analysis was performed initially with different chemical reagents to detect the nature of phytoconstituents and their presence in plant parts. The presence of sterols/terpenes, flavonoids, tannins, alkaloids, lignins, saponins and cumarins were evaluated by standard qualitative methods [5, 6].

##### 2.2.2. Determination of Chemical Constituents of the Plant Extracts Using GC-MS

The plant extracts were dissolved in ethanol (HPLC Grade) then the solution was transferred into the vial by syringe and micro-filter to avoid the presence of any particles not dissolved by the solvent, then the vial was inserted in the device. The qualitative and quantitative analysis of the sample was carried out by using GM/MS technique model (GC/MS-QP2010-Ultra) from Japan Shimadzu Company, with serial number 0205251015S5A and capillary column (Rtx-5ms-30m×0.25 mm×0.25µm). The sample was injected by using split mode, helium as the carrier gas passed with flow rate 1.61 ml/min, the temperature program was started from 60°C with rate 10°C /min to 300°C as final temperature degree with 2 minutes hold time, the injection port temperature was 300°C, the ion source temperature was 200°C and the interface temperature was 250°C. The sample was analyzed by using scan mode in the range of m/z 40-500 charges to ratio and the total run time was 26 minutes. Identification of components for the sample was achieved by comparing their retention times and mass fragmentation patterns with those available in the library, the National Institute of Standards and Technology (NIST) [7].

#### 2.2.3. Antioxidant Activity

The DPPH radical scavenging was determined according to the method of Shimada et al [8] with some modification. In 96-wells plate, the test samples were allowed to react with 2,2Di (4-tert-octylphenyl)-1-picyl-hydrazyl stable free radical (DPPH) for half an hour. The concentration of DPPH was kept as (300 µl). The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517 nm using Shimadzu Spectrophotometer UV double beam. Percentage radical scavenging activity by samples was determined in comparison with DMSO treated control group. Ascorbic acid was used as standard. The ability to scavenge the DPPH radical was calculated using the following equation: DPPH scavenging activity (%) = (A0 – A1) /A0 ×100.

Where A0 is the absorbance of the control and A1 is the absorbance of the sample [9].

### 3. Results and Discussions

#### 3.1. Qualitative Preliminary Phytochemical Analysis

Plants are composed of secondary metabolites which can be detected through phytochemical screening, which is the process of tracing these secondary metabolites in plants. Secondary metabolites are organic compounds which are not involved in the process of growth, development, reproduction of an organism, or other primary functions. Therefore in the absence of these compounds a plant will not die immediately, but will result in long term impairment in other functions, such as defensive mechanisms or aesthetic appearance, or in some cases of impact. Where a primary metabolites are necessary for functioning of the plant. Extracts of the plant were prepared using the powdered form and an appropriate solvent. The extract was then subjected to a range of chemical tests to detect the presence or absence of the secondary metabolites in the plant material. Qualitative preliminary phytochemical analysis was performed initially with different chemical reagents to detect the nature of phytoconstituents and their presence in bark and leaves extracts of *K. senegalensis*. Results of the presence of primary and secondary metabolites showed in Table 1. Phytochemical screening showed that the leaves and bark extracts were rich in chemical constituents. Both extracts contained phenolic compounds and fatty acids which are found to have anti diabetic activity. The results of chemical constituents of *K. senegalensis* extracts agree with that obtained by Elish et al. who reported that *K. senegalensis* contain secondary metabolites such as cumarins, tannins, compounds reducers,
Table 1. Preliminary phytochemical screening of leaf and bark extracts of 
khaya senegalensis.

| Test       | Specific test | Sample       | Leaf extract | Bark extract |
|------------|---------------|--------------|--------------|--------------|
| Alkaloids  | Wagner’s       | -ve          | -ve          | -ve          |
|            | Mayer’s       | +ve          | -ve          | -ve          |
|            | Dragendorf’s  | +ve          | -ve          | -ve          |
| Flavonoids | FeCl3         | +ve          | -ve          | -ve          |
| Sterols    | Lead acetate  | +ve          | -ve          | -ve          |
| Salkowski  | +ve           | -ve          |              |              |
| Libermann  | +ve           | -ve          |              |              |
| Triterpines| Salkowski     | +ve          | -ve          | -ve          |
|            | +ve           | -ve          |              |              |
|             | -ve           |              |              |              |
| Tannins    | Gelatin       | -ve          | +ve          | -ve          |
|            | HNO2         | -ve          | +ve          | -ve          |
|            | Lead acetate  | -ve          | +ve          | -ve          |
| Saponins   | Foam test     | +ve          | +ve          | -ve          |
| Cumarins   | UV test       | -ve          | +ve          | -ve          |
| Glycosides | kellkiliani   | +ve          | -ve          | -ve          |
| Reducing sugar | keld’s     | -ve          | +ve          | -ve          |
| Anthraquinones | Fehling’s   | -ve          | +ve          | -ve          |
| Lignins    | Labat test    | -ve          | +ve          | -ve          |
| Carbohydrates | Molish       | +ve          | +ve          |              |

Table 2. Chemical composition of leaves ethanol extract of khaya senegalensis using GC-MS.

| NO. | Name of Compound                  | Ret. Time | Area% |
|-----|-----------------------------------|-----------|-------|
| 1   | Benzyl chloride                   | 4.693     | 0.28  |
| 2   | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | 6.662     | 0.41  |
| 3   | Catechol                          | 7.902     | 4.22  |
| 4   | 2-Methoxy-4-vinylphenol           | 9.105     | 1.10  |
| 5   | Phenol, 2,6-dimethoxy             | 9.621     | 1.05  |
| 6   | Phenol, 2-chloro-5-methyl         | 10.214    | 2.18  |
| 7   | 1,3-Propanediol, 2-ethyl-2-(hydroxymethyl) | 10.908    | 4.66  |
| 8   | beta-D-Glucopyranose, 1,6-anhydro | 11.583    | 1.40  |
| 9   | 2-(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl | 12.131    | 0.16  |
| 10  | Ethyl iso-allocloholte             | 12.308    | 0.74  |
| 11  | 4-O-Methylmannose                 | 13.814    | 14.73 |
| 12  | 3,7,11,15-Tetramethyle-2-hexadecen-1-ol | 15.463    | 9.62  |
| 13  | 2-Hexadecene, 3,7,11,15-tetramethyl | 15.535    | 0.76  |
| 14  | 9-Eicosyone                       | 15.734    | 1.61  |
| 15  | Phytol, acetate                   | 15.935    | 3.14  |
| 16  | Albuterol                         | 16.412    | 0.90  |
Table 3. Chemical composition of bark ethanol extract of K. senegalensis using GC-MS.

| NO. | Name of Compound                                      | Ret. Time | Area% |
|-----|-------------------------------------------------------|-----------|-------|
| 17  | n-Hexadecanoic acid                                   | 16.806    | 7.99  |
| 18  | Hexadecanoic acid, ethyl ester                        | 17.094    | 2.69  |
| 19  | Arabino-Hex-1-enitol, 1,5-anhydro-2-deoxy-            | 17.415    | 3.01  |
| 20  | Phytol                                                | 18.334    | 4.70  |
| 21  | 9,12,15-Octadecatrienoic acid, (Z, Z, Z)-             | 18.631    | 1.13  |
| 22  | 9,12-Octadecadienoic acid (Z, Z)-                     | 18.786    | 1.45  |
| 23  | Ethyl 9,12,15-octadecatrienoate                       | 18.860    | 1.60  |
| 24  | Octadecanoic acid, ethyl ester                        | 19.042    | 1.14  |
| 25  | Squalene                                              | 24.346    | 14.53 |
| 26  | gamma.-Tocopherol                                     | 26.538    | 0.99  |
| 27  | Vitamin E                                             | 27.441    | 13.81 |

Figure 1. GC-MS chromatogram of leaves ethanol extract of K. senegalensis.

Figure 2. GC-MS chromatogram of bark ethanol extract of K. senegalensis.
The utilization of this plant for traditional medicine by the people also predicted that the plant could have some bioactive ingredients. The present study was similar with that obtained by Aguoru [11] who reported the methanolic and aqueous extracts of stem bark of *Khaya senegalensis* had many chemicals identified using retention time, relative percentage of the compound, molecular weight and molecular formula included: pentadecanoic acid, n-hexadecanoic acid, 9, 12-octadecadienoic acid, and 11-octadecenoic acid. Others were 9-hexadecenoic acid, stearic acid, hexadecanoic acid, ricinoleic acid and 13-decosenoic acid. Some of these chemicals especially the acids have been demonstrated to be bioactive [19, 12].

3.3. DPPH Radical Scavenging Activity

In-vitro antioxidant activity of the ethanolic extracts of leaves and bark from *k.senegalensis* was evaluated using DPPH assay; results are shown in Table 4. The *in vitro* antioxidant activity of the leaves and bark extracts of *K.senegalensis* was evaluated using DPPH assay. Results in Table 4 showed the highest result of antioxidant activity by DPPH scavenging assay in leaves extract (42.69%) and lower
result shown in bark extract (36.36%). In comparison with ascorbic acid which gave 93.5% the leaves extract showed moderate activity but bark extract showed low activity. Previous study on K.senegalensis revealed that leaves and bark have potent antioxidant activity, the results agree with that obtained by Ibrahim who reported that the highest scavenging activity was observed with the ethanolic extract of the root then leaves followed by the bark aqueous extract [17]. The higher activity of leaves may be due to the presence of Vitamin E in this extract because it has free radical scavenging activity and present with high percentage of (13.81%). Antioxidant capabilities and free radical scavenging properties are essential in using plant materials for curative purposes. The extracts from the three parts of the plant have exhibited antioxidant capabilities in comparison with the standard ascorbic acid [20].

Table 4. Antioxidant activity of leaves and bark extracts of k.senegalensis.

| Sample               | DPPH% |
|----------------------|-------|
| Leaves extract       | 42.69 |
| Bark extract         | 36.36 |
| Ascorbic acid (Standard) | 93.5  |

Figure 5. Comparison between DPPH scavenging activity of Ascorbic acid and K.senegalensis extracts.

4. Conclusion

Khaya senegalensis (African mahogany) is medicinally important and used for various purposes. Phytochemical screening showed that the leaves and bark ethanolic extracts were rich of chemical constituents, the presence of these secondary metabolites validate the use of the plant as herbal drug in Sudan. The study of chemical composition may possess significant bioactive compounds which contributed to the pronounced antioxidant and other biological activity.

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