

## Phytochemical Study from Aqueous Extracts of Indigenous Medicinal Plants against from Diabetes in the Peyrie Market in Gabon

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### ABSTRACT

Diabetes mellitus management is not well established especially in Gabon. More than 60% of population relies on traditional treatments as primary healthcare in Africa. This paper covers the preliminary investigation of different phytochemicals. A total 130 tests were investigated with 85 (65,38%) positive and 50 (34,62%) negative. The phytochemical screening of the aqueous extracts of the indigenous plant revealed the presence of alkaloids, flavonoids, tannins (catechic or gallic), quinones, triterpenes, carotenoids, carbohydrates, mucilages, reducing sugars and saponosides at varying amount depending on the intensity of the colors observed. Steroids were absent in these extracts. Alkaloids, carbohydrates, reducing sugars, flavonoids, gallic tannins, quinones and triterpenes constitute important group. The presence of variable compounds suggests that this plants has medicinal value and their used from diabetes treatment. Further, chromatographically investigations have necessary for determinate structure and natural compounds responsible for activities.

**Keywords:** medicinal plants, phytochemical compounds, diabetes, Gabon.

### INTRODUCTION

Diabetes mellitus is metabolic health trouble which increasing in the world. One person is diagnosed with the diabetes every 5 seconds all around the world and someone dies from it every 10 seconds<sup>1</sup>. International Diabetes Federation (IDF) projected that the pandemic would spend from 371 million in 2011 to 552 million 2030<sup>2,3</sup>. In added, the World Health Organization (WHO) considers in 2000 that the number of diabetics in Africa would cross from 14 million to 30 million in 2025, and says that this disease will be the 7<sup>th</sup> foremost worldwide cause of death in 2030<sup>4</sup>. Gabon is the third country in Sub-Saharan affected with of more than 9% of the registered cases<sup>5</sup>. Conventional drugs used against diabetes are associated with several adverse effects<sup>6</sup>. Diabetes is a costly disease for the individual, the family and the community. The prohibitive costs to the poor, guide most patients to traditional remedies or to combine the two medicines<sup>7</sup>. More than 20,000 medicinal plants are known in Africa, but less than 1% of them were scientifically investigated<sup>8</sup>. Besides, several ethnobotanical studies provide are used against diabetes, but little were investigated, as the metformin derived of guanide which was obtained from *Galega officinalis* L. (Fabaceae)<sup>6</sup>. In spite of the increasing use of medicinal plants, there is important lack of data research in this domain. To overcome the risks of overdose, poisoning or placebo, it is urgent to double efforts to update new medicine. WHO encourages the development of the medications with plants which it considers as candidates

for the excellent oral therapy<sup>9</sup>. The study of natural substances from plants used in various traditional medicines around the world to fight against such infections, or synthetic products derived from these substances are outstanding a way of exploration.

Phytochemicals constituents were used as drug and remedies for diverse diseases for a long period, example: anti-inflammatory, antioxidants, antimicrobial, hepatoprotective<sup>10,11,12,13</sup>. Phytochemistry screening is a process which supplies information on him or chemical groups of natural substance. This process would allow directing the use of aforementioned natural with the traditional healers. Our study focus the detection of the present secondary metabolites in 10 spontaneous medicinal plants traded on the Peyrie market to Libreville in Gabon against the diabetes.

### MATERIALS AND METHODS

#### Materials

The plant material is composed of the barks of *Picalima nitida*, *Alstonia congensis*, *Annickia chlorantha*, *Greenwayodendron suaveolens*, *Guibourtia tessmannii*, *Anthochleista vogelii*, *Cylicodiscus gabunensis* and *Hallea ledermannii*. The roots of *Strychnos icaia* and the whole plant of *Momordica charantia*.

The reagents used for the characterization of the different phytochemical constituents are: hydrochloric acid, sulfuric acid, acetic anhydride, ammonia 30%, chloroform, concentrated sulfuric acid, alcohol isoamyl, formic acid,

Table 1: Chemical groups, identification and indicator.

| Chemical compounds    | Reagents                                                                                                               | Indicator                                                                           |
|-----------------------|------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| Alkaloids             | Dragendorff (tetraiodo -bismuthata of potation)<br>Mayer (mercuri-iodure of potacium)                                  | range precipitate<br>White Precipitate                                              |
| Flavonoïds            | Cyanidine reaction                                                                                                     | Red cherry (flavanol)<br>Orange (flavone)<br>Purplish red (flavone)                 |
| Quinones              | Concentred ammonia and soda 25%                                                                                        | Orange-red<br>Red<br>Purplish                                                       |
| Tannins               | 10% Ferric chloride reaction                                                                                           | Brown-green precipitate (tannin catechin)<br>Blue-black precipitate (gallic tannin) |
| Sterols / Triterpenes | Lieberman-Burchard reaction (acetic anhydride, sulfuric acid)                                                          | Green (sterols)<br>Purplish red (triterpenes)                                       |
| Carotenoïds           | Chloroform, sulfuric acid                                                                                              | Blue or purplish                                                                    |
| Sugar                 | Molish reaction (alpha-naphthol, ethanol, sulfuric acid)                                                               | Purplish                                                                            |
| Reducing sugar        | Fehling reaction (copper sulphate, (Seignette salt = sodium and potassium tartate, sodium hydroxide), distilled water) | Precipitated brick red                                                              |
| Mucilage              | Ethanol                                                                                                                | Brown flake                                                                         |
| Saponosides           | Foam index                                                                                                             | Persistent foam                                                                     |

sodium hydroxide, alpha-naphthol and 1% ferric chloride (Merck, Carlo Erba, Normapur). Specific reagents commercially available have been used: the reagents of Dragendorff, Mayer and Fehling (Carlo Erba, Prolabo). Fragments of magnesium chips were also used (Sigma). Reagents for the chemical compounds identification are mentioned in table 1 with the specific indicators.

#### Preparation of aqueous extracts

Selected parts of plants which indicated during the survey were dried using a dehumidifier (Bioblock Scientific LMS Cooled Incubator) at 35°C for one week and crushed to powder. 10g of each sample have been boiled at 100°C with 100ml of distilled water for 1h, after cooling the decoction have filtered and preserved for the chemistry tests.

#### Phytochemistry screening

The detection of secondary metabolites in the samples was carried out according to the interpretation technic of reactions. To the end, various known reagents are added to the samples and the quality of the reaction obtained is possible to conclude as to the presence of the desired compound or not. According to the precipitation or color intensity in each sample and tests, following evaluations were given: (+) for presence and (-) for absence. Qualitative chemical constituents were screened according standard methods of our laboratory as Mengome et al<sup>14</sup> or Bajin ba Ndob et al<sup>15</sup>. The identification of the following groups was considered: alkaloids, sterols and triterpenes, flavonoids, tannins, quinones, carotenoids, reducing sugars, carbohydrates and saponosides.

**Alkaloids:** 2 ml of each aqueous extract was agitated with 5 ml of hydrochloric acid in as team bath, then, 1ml aliquots of filtrate were treated with a few drops of Mayer's reagent or Dragendorff's reagent. The presence of a precipitate after treatment with either reagent is a

preliminary indicator of the presence of alkaloids. To remove non-alkaloid compounds that could lead to false-positive reactions, part of the extract was alkalized with 30% ammonia solution then treated with chloroform. The second chloroform extract was concentrated and then retested with the Mayer's and Dragendorff's reagents. **Flavonoids** were detected by using the Shibata reaction or cyanide test. Briefly, 2 ml of each aqueous extract were added in 2 ml of 50% methanol, then, a few magnesium shavings and a few drops of concentrated hydrochloric acid were added. The development of a red-orange or purplish color indicates the presence of flavones aglycones. **Tannins:** 2 ml of each aqueous extract were mixed with 1% ferric chloride. A black-blue color indicates the presence of gallic tannins and a dark green color tannin catechits. When both were detected in the same extract, they were separated with Styasny's reagent. A drop of the extract was placed on as lab of silica gel and eluted in an atmosphere saturated with chloroform/aceticacid/formic acid (5:4:1). The plates were sprayed with 10 ml of methanol solution at 5% nitrous acid and heated in an oven at 80°C for 10 min. The presence of tannins is revealed by the appearance of blue spots, while polyphenols are revealed by a violet-blue, pink-orange, pink-violet, or red coloration. **Quinones:** they are identified by Bornstraëgen reagent. Briefly, 2 ml of aqueous extract are added to 5 ml of hydrochloric acid in the water bath for 30 minutes, after cooling, 10 ml of chloroform are added and 0.5 ml of ammonia. Red or violet coloration indicates the presence of quinones. **Sterols or triterpenes:** these families of compounds were identified by using the Lieberman-Burchard reaction. Briefly, 2 ml of each aqueous extract were added in 0.5 ml of chloroform, 0.5 ml of acetic anhydride, and cooled on ice before carefully adding sulfuric acid. The changing color

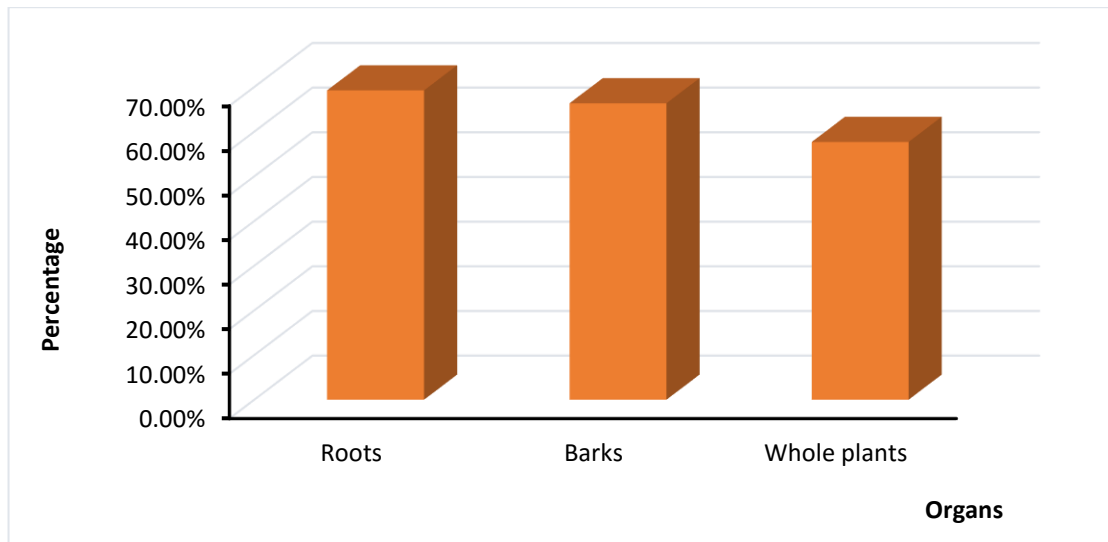


Figure 1: Organs richness.

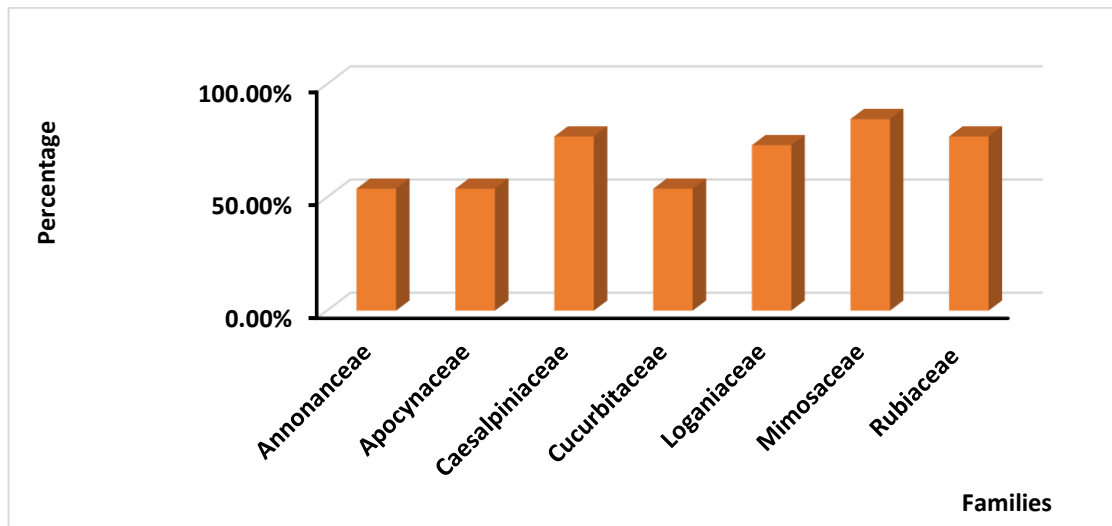


Figure 2: Categories families according richness of chemical compounds.

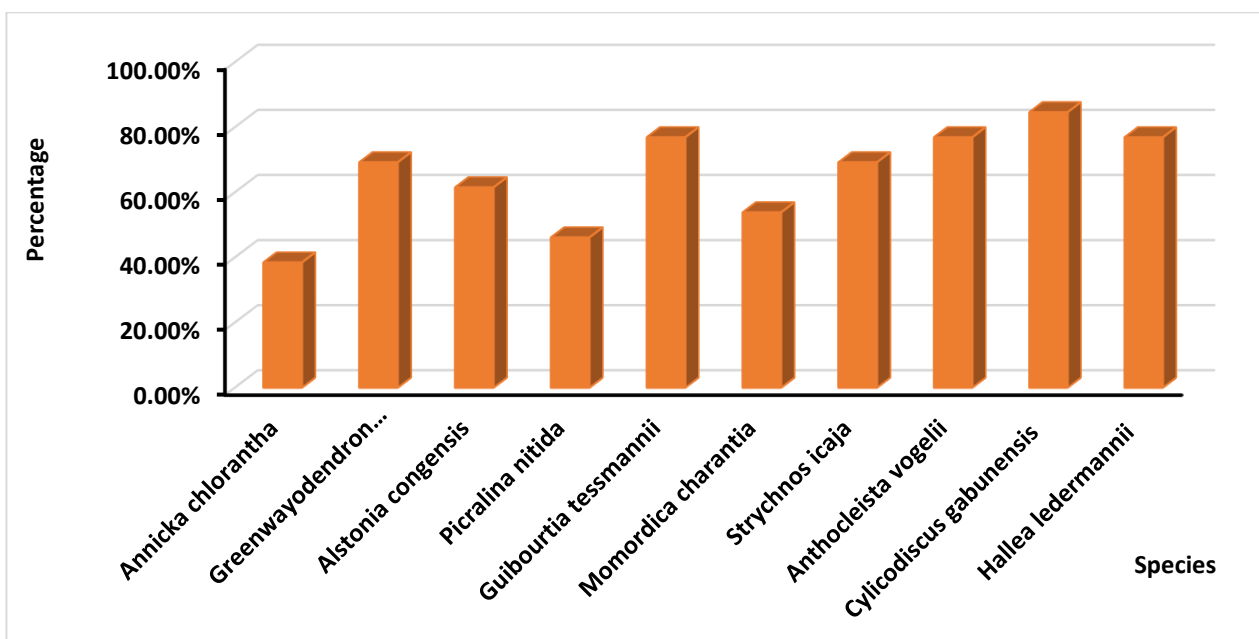


Figure 3: Categories plants studies according to their abundance of chemical compounds.

from purple to blue indicates the presence of sterols, while a green or purple–red color indicates the presence of triterpenes. *Carotenoids*: 2 ml of each aqueous extract were added with a few drops of concentrated sulfuric acid. The changing blue to red indicates the presence of carotenoids. *Carbohydrates*: 2 ml of each aqueous extract were added with few drops of Molish solution, allowed turn on the tube wall few drops of concentrated sulphuric acid. Carbohydrate presence was detected by the emergence of a violet ring at the interface. *Reducing sugars*: 2 ml of each aqueous extract were added in 1 ml of Fehling liquor and boiled for 30 min. The formation of a brick-red precipitate indicates the presence of reducing sugars. *Saponosides*: Each sample decoction (1%) was returned gradually in 10 ml test tubes for a final volume of 10 ml. After two vigorous shakes, the tubes were left to stand for 15 min and the height of foam was measured. The tube in which the height of the foam was at least 1 cm, showed the presence of saponosides. However, the height of the foam indicated the value of the foam index.

## RESULTS AND DISCUSSION

Global qualitative screening results of chemical groups for the aqueous extracts from plants studies (Table 2) showed alkaloids, flavonoids, tannins (gallic or catechic), quinones, triterpenes, carotenoids, carbohydrates, reducing sugars, mucilages and saponosides.

These substances could be to the origin of the pharmacological effects of these plants used in traditional medicine to treat many diseases such as diabetes. Several studies have revealed the presence of some of the constituents identified by way of is the case of *Cylicodiscus gabunensis*<sup>16,17</sup>; *Picalima nitida*<sup>15</sup>; *Guiburtia tessmannii*<sup>18</sup>; *Annickia chloranta*<sup>15,19</sup>; *Greenwayodendron suaveolens*<sup>14</sup> with some differences. Sterols have not revealed in this study, although some studies reveal their presence in *Cylicodiscus gabunensis*<sup>16,17</sup>; *Anthocleista vogelii*<sup>20</sup>, *Guiburtia tessmannii*<sup>18</sup> and *Annickia chloranta*<sup>21</sup>. Globally, 130 tests were investigated with 85 (65,38%) are positive and 45 (34,62%) are negative.

The observation of Figure 1 shows that the roots are the richest in chemical substances. Out of a total of 13 tests, 9 are positive, a rate of 69,23%. Following from the barks with 69 out of 104 positive cases through 66,35%. Finally, the whole plant out of the 26 tests performed, 15 are positive and given 57,69%. These results can be justified by the fact that the roots are the place of reserve for plants<sup>22</sup>. However, all plant parts used are rich in chemical substances which may justify their use in traditional medicine against the treatment of diabetes.

Abundance of chemical compounds could be assessed by family group on figure 2. Four groups were observed and chemical compounds are strongly. The first group is the Mimosaceae family with a rate of 84,62%. The second includes two families (Caesalpinaceae and Rubiaceae), with a rate of 76,92% each. The third group contain Loganiaceae family with 73,08%. The fourth group includes three families (Annonaceae, Apocynaceae and Curcubitaceae) with 53,85% each. Several pharmacological studies have shown activities in these families. Antiproliferative effects of colon cancer cells (CaCo-2) of the alcoholic extract for *Piptadeniastrum africanum* roots a Mimosaceae have been shown<sup>14</sup>. Anti-diabetic activity showed of the leaves for *Dialium guineense* Wild a Caesalpinaceae<sup>23</sup>.

As seen in figure 2, the abundance of chemical compounds is consistent in figure 3 with reference to each species studied. Of the ten (10) plants studies, eight (8) are very rich in compounds, ranging from 84,62% to 53,85%. They can be classified into four groups in order of richness. The first group has four species that are *Cylicodiscus gabunensis* with 11 types of chemical compounds is a rate of 84,62%. *Anthocleista vogelii*, *Hallea ledermannii* and *Guiboutia tessmannii* with 10 types of compounds is a rate of 76,92%. The second group has two species that are *Greenwayodendron suaveolens* and *Strychnos icaja* with 9 types of compounds a rate of 69,23%. The third contains two species that are *Alstonia congensis* and *Momordica charantia* with a rate of 61,54% and 53,85% respectively. Finally, the fourth group which contains less rich species, it includes *Picalima nitida* and *Annickia chlorantha* with

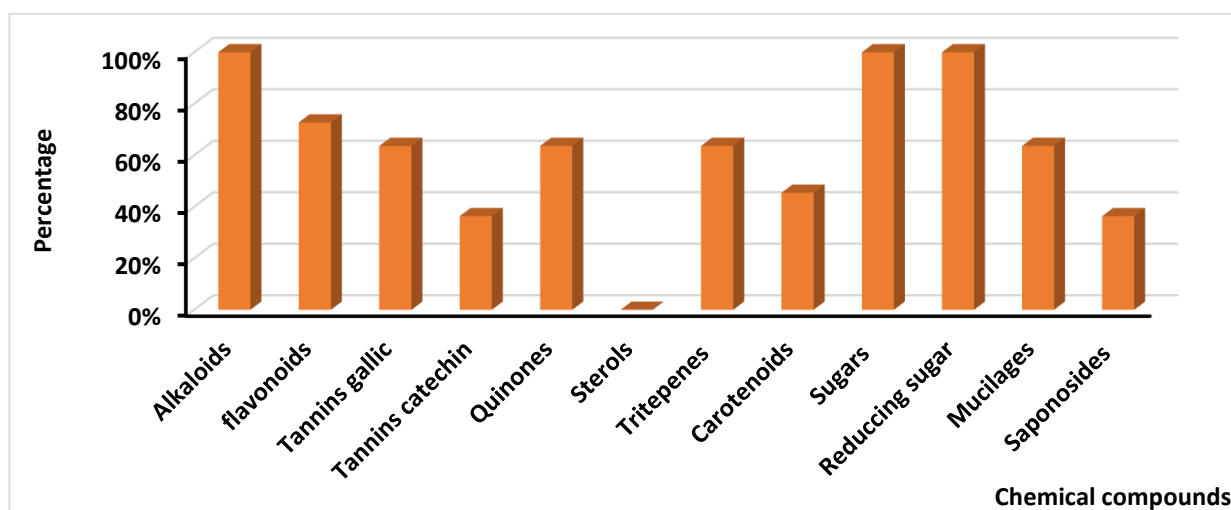


Figure 4: Categories chemical compounds according to their abundance.

Table 2: Phytochemistry screening some species used to treat diabetes in Peyrie market in Gabon.

| Families        | Scientific name                                            | Extracts | AlkD | AlkM | Fla | Tang | Tanc | Qun | Ste | Trit | Car | Sug | Res | Muc | Sap | Org | R+             |
|-----------------|------------------------------------------------------------|----------|------|------|-----|------|------|-----|-----|------|-----|-----|-----|-----|-----|-----|----------------|
| Annonaceae      | <i>Annickia chloranta</i> (Oliv.) Setten & Mass            | dia-AnCE | +    | +    | -   | +    | -    | -   | -   | -    | -   | +   | +   | -   | -   | B   | 5<br>(38.46%)  |
|                 | <i>Greenwayoden dron suaveolens</i> (Engl. & Diels) Verdc. | dia-GrSE | +    | +    | +   | +    | -    | +   | -   | +    | -   | +   | +   | +   | -   | B   | 9<br>(69.25%)  |
| Apocynaceae     | <i>Alstonia congensis</i> Engl.                            | dia-AICE | +    | +    | +   | +    | -    | +   | -   | +    | -   | +   | +   | -   | -   | B   | 8<br>(61.54%)  |
|                 | <i>Picralima nitida</i> (Stapf) T. Durand & H. Durand      | dia-PiNE | +    | +    | -   | +    | -    | +   | -   | -    | -   | +   | +   | -   | -   | B   | 6<br>(46.15%)  |
| Caesalpiniaceae | <i>Guiburtia tessmannii</i> (Harms) J. Leonard             | dia-GuTE | +    | +    | +   | +    | -    | +   | -   | +    | +   | +   | +   | +   | -   | B   | 10<br>(76.92%) |
| Cucurbitaceae   | <i>Momordica charantia</i> L.                              | dia-MoCP | +    | +    | -   | -    | +    | -   | -   | +    | -   | +   | +   | -   | +   | Wp  | 7<br>(53.85%)  |
| Loganiaceae     | <i>Strychnos icaja</i> Baill                               | dia-StIR | +    | +    | +   | -    | +    | -   | -   | -    | +   | +   | +   | +   | +   | R   | 9<br>(69.23%)  |
|                 | <i>Anthocleista vogelii</i> Planch.                        | dia-AnVE | +    | +    | +   | +    | -    | -   | -   | +    | +   | +   | +   | +   | -   | B   | 10<br>(76.92%) |
| Mimosaceae      | <i>Cylocodiscus gabunensis</i> Harms                       | dia-CyGE | +    | +    | +   | -    | +    | +   | -   | +    | +   | +   | +   | +   | +   | B   | 11<br>(84.62%) |
| Rubiaceae       | <i>Hallea ledermannii</i> (K. Krause) Verdc.               | dia-HaLE | +    | +    | +   | -    | +    | +   | -   | +    | -   | +   | +   | +   | +   | B   | 10<br>(76.92%) |

AlkD: Alkaloids according Dragendorff test, AlkM: Alkaloids according Mayer test, Fla: Flavonoids, Tang: Tannins gallic, Tanc: Tannins catechic, Qun: Quinones, Ste: Steroids, Trit: Triterpenes, Car: Carotenoids, Sug: Total sugars, Res: Reducing sugars, Muc: Mucilages, Sap: Saponosids, Org: organs, R+: positive results tests, B: Barks, Wp: Whole plant, R: Roots.

a rate of 46,15% and 38,46% respectively. Several data from the literature reveal the pharmacological activities of these species.

On 13 compounds examined for each plant, 9 are plentiful (figure 4) and several studies confirm that some of these compounds have activities on the diabetes. This figure illustrated a high concentration in all extracts by alkaloid, carbohydrate and reducing sugar (100%). The presence of carbohydrates in all extracts may be explained by their intervention in the building of the plant and because they are the precursors of secondary metabolites concentrated in the parts of plants. Several studies related pharmacological effect of the carbohydrates, alkaloids and others second metabolites. Indeed, previous studies show that, a water-soluble polysaccharide extract would increase reduce blood glucose level, increase the insulin level and remediating destruction of pancreatic islets in STZ-induced diabetic rats<sup>24</sup>. Alkaloids isolated in *Catharanthus roseus* and a gluco-alkaloid: solanine isolated in *Solanum amaricanum* has showed hypoglycemic effects<sup>25</sup>. Ibogaine indole alkaloids from *Tabernanthe iboga* increased beta-cells insulin<sup>26</sup>. Akuammicine an alkaloid from chloroform

extract of *Picralima nitida* seeds would stimulated the increase in glucose uptake in fully differentiated 3T3-L1<sup>27</sup>. Acetone extract leaves of the same plant would show inhibition of alpha-amylase and alpha-glucosidase activities<sup>28</sup>. Antidiabetic activity of methanol extract of *Picralima nitida* leaves have showed by Teugwa et al.<sup>29</sup>. Mixture of hydroalcoholic extract of *Alstonia congensis* bark and *Xylopiya aethiopia* fruit would reduce blood glucose levels in Swiss albinos rats<sup>30</sup>. Flavonoids are the second most abundant group in this study. They are known to have hypoglycaemic and antidiabetic activities<sup>31,32,33,34</sup>. Flavonoids are excellents antioxidant which is beneficial against diabetes troubles due to oxidative stress state<sup>35</sup>. These compounds would inhibit alkalose reductase<sup>36</sup>. Flavonoids would also produce an antihyperlipidemic effect by decreasing the concentration of fatty acids in the circulation as well as reducing cholesterol synthesis<sup>37,38</sup>. Quercetin isolated from *Psidium guajava* increase glucose uptake in hepatocytes<sup>39</sup>. Gallic tannins, quinones and triterpenes constitute the third important group. Several studies recognized antidiabetic from these compounds such as tannins isolated from *Ficus*

*racemosa* would attenuated oxidative stress, would ameliorated hyperglycemia and dyslipidemia in diabetic rats<sup>40</sup>. Tannins would be excellents regulators of cholesterol in the blood also<sup>41</sup>. We would suggest that these metabolites may promote insulin receptor sensitivities or stimulate Langarhans island beta cells to release insulin to enhance carbohydrate metabolizing enzymes toward normal blood glucose recovery.

## CONCLUSION

The presence of alkaloids, flavonoids, tannins (gallic or catechic), triterpenes, carotenoids, mucilages, cabohydrates, reducing sugars and saponosides can be have benefic, because their properties are responsible for the pharmacological effects from mainly research's. At the further, it is necessary that natural extracts are processed and standardized as herbal formulation which will be commercialized and used in clinical sittings.

These natural compounds or their active ingredients can help evaluate the beneficial effects of diabetes by evaluating compounds that have already been studied to produce new antidiabetic drugs accessible for all.

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