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Phytochemical investigation of five herbal teas useful in mental disorders treatment

Jules Yoda, Benjamin Ouédraogo, Sosso Siaka, Félix B Kini, Abdoulaye Djandé and Adama Saba

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Abstract
Mental disorders or illnesses are common and affect all countries. According to official estimates, in most countries at least one person in three is affected. Several researches have shown the benefits of plants in the management of these pathologies. This study focuses on five medicinal plants useful in the management of mental disorders, including Cymbopogon citratus, Lippia chevalieri, Combretum micratum, Chrysantellum americanum and Tetrapleura tetraptera. These plants are used in Burkina Faso in the form of herbal teas. The objective of this study is to enhance the value of these plants and improve their use by some physico-chemical analyses, a phytochemical study and the determination of antioxidant activity. Among the parameters that will be precisely investigated there are organoleptic analysis, moisture content, extraction yields, thin layer chromatographic fingerprinting, phytochemical screening and the determination of total phenol, total flavonoids. The DPPH method was used to evaluate the antioxidant power of the various aqueous extracts. The physico-chemical analysis gave satisfactory results on the main indices. The phytochemical study allowed to establish the chromatographic fingerprints of the aqueous extracts, to identify the main bioactive secondary metabolites and to determine the content of total phenols and total flavonoids in the extracts of the five plants. Interesting results are obtained, which confirms the use of these plants. Among the samples submitted to the different analyses, the extract of Combretum micratum presented high levels of total phenolics (395.38 ± 7.05 mg GAE/g extract) and total flavonoids (105.87 ± 0.05 mg Ru E/g extract). This extract also exhibited the highest antiradical activity.

Keywords: Mental disorders, herbal teas, phytochemical screening, phenolic compounds, antiradical properties

1. Introduction
Mental disorder or mental illness is a behavioural pattern that results in impaired personal functioning [1]. Common mental disorders generally refer to two broad categories of diagnoses including depressive disorders and anxiety disorders. These conditions are among the most common causes of morbidity and disability worldwide [2, 3]. According to estimates, the most common mental disorders are depression, which affects about 264 million people, bipolar disorder, which affects about 45 million people, dementia, which affects about 50 million people, and schizophrenia and other psychoses, which affect about 20 million people worldwide [4]. The causes of these diseases are often multiple and confusing, which makes the management by modern medicine difficult. In some countries, phytotherapy is found as a safe alternative in the treatment of several mental illnesses [5]. Despite the great advances in modern medicine in recent years, especially in the field of organic synthesis of bioactive molecules, plants still contribute significantly to the improvement of the health of the world population, especially in underdeveloped countries [6, 7]. Plants, with their wide variety of chemical constituents, offer a promising source of medicines for many diseases, including mental disorders. This present study concerns Cymbopogon citratus, Lippia chevalieri, Combretum micratum, Chrysantellum americanum and Tetrapleura tetraptera involved in the treatment of mental illness [8, 9]. These five plants have been used as herbal teas for years in some West African countries, i.e. Burkina Faso. The objective of this study is to contribute to the valorization of these medicinal plants through chemical investigations such as chromatographic fingerprinting, phytochemical screening and phenolic compound content. Antioxidant activity was also evaluated using antiradical test with DPPH reagent.
2. Materials and Methods

2.1. Sample Collection and Preparation
The plant material consisted of organs of the five species including the leaves of *Cymbopogon citratus*, *Combretum micranthum*, *Lippia chevalieri*, the whole plant of *Chrysantellum americanum* and the the fruits of *Tetrapleura tetraptera*. These plant parts were collected in October 2019 in two locations. They were dried away from light, moisture and dust. They are then pulverized by means of a blade mill and the powders thus obtained will be used to carry out the various extraction operations.

2.2. Organoleptic Tests
The examination of organoleptic characteristics includes analyses of the general shape, colour, smell of the drug [14].

2.3. Moisture Content
A test sample is weighed and the resulting mass is recorded. The drying process is repeated until the moisture mass of 1g of powder sample was placed a in crucible and dried in an oven at 105 °C. After cooling, the steamed content attained a constant value [15].

2.4. Extraction Yield
The extracts used for phytochemical screening are obtained by depletion of the different crude aqueous extracts with solvents in order of increasing polarity (n-hexane, dichloromethane, ethylacetate, butanol) [14]. For the other analyses, the extracts were obtained by aqueous decoction.

2.4. Fingerprinting by Thin Layer Chromatography
Each sample was solubilized (10 mg/mL) and 5 µL was deposited on a 60 F254 silica gel plate (glass support). The chromatographic plate is then immersed in the tank containing the desired mobile phase for the development of the chromatogram over a distance of 8 cm. After the chromatogram has been developed, the chromatographic plate is visualized using a UV lamp. The TLC was conducted according to the general methods of the Pharmacopoeia.

2.5. Determination of the Phytochemical Constituent
The phytochemical screening of the plants extracts was carried out by simple qualitative methods [16].

2.6. Total Phenolics Content
Total phenolic content were determined by Singleton method [17]. These compounds react with the Folin Ciocalteu reagent (FCR) in an alkaline medium. The loss of a phenolic proton in the FCR in molybdate forming a blue colored complex with a maximum absorption at 760 nm. The intensity of the blue coloration is proportional to the amount of total phenolics present in the test sample. The reaction mixture consisted of 1 ml extract, 1 ml 2N FCR and 3 ml 20% sodium carbonate solution. It was left at rest at room temperature for 40 min and then the absorbance is measured at 760 nm on the spectrophotometer (Agilent 8453). In the white control tube, the extract was replaced by distilled water. A standard curve will be drawn with tannic acid (1-5 mg/mL).

2.7. Total Flavonoids Content
The flavonoid assay was performed using the Kumaran method [18] adapted by Abdel-Hameed [19]. 2 ml of extract of concentration 1 mg/ml in methanol were mixed with 2 ml of 2% aluminium trichloride in methanol. After 40 minutes, the absorbance was measured at 415 nm using the spectrophotometer (Agilent 8453). The white control tube consisted of 2 ml of methanol and a standard curve was plotted with quercetin. The tests were carried out in triplicate.

2.8. Determination of Antiradical Properties
The technique used to determine the antiradical activity of extracts is based on the methodology developed by Takeo with slight modifications [20]. 1,1-diphenyl-2-picrylhydrazyl (DPPH), the reference oxidant, is dissolved in methanol at a concentration of 2 mg/mL and used to spray TLC plates after migration.

3. Results and Discussion
3.1. Organoleptic test and moisture content
To obtain the organoleptic characteristics, we determined the main indices such as color, odor and flavor of the raw powder of each of the five plants. The results are recorded in Table 1.

<table>
<thead>
<tr>
<th>Crude powder</th>
<th>colour</th>
<th>odour</th>
<th>Moisture Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cymbopogon citratus</em></td>
<td>greenish</td>
<td>citrus scent</td>
<td>7,39</td>
</tr>
<tr>
<td><em>Lippia chevalieri</em></td>
<td>greenish</td>
<td>odourless</td>
<td>6,99</td>
</tr>
<tr>
<td><em>Combretum micratum</em></td>
<td>greenish</td>
<td>citrus scent</td>
<td>3,94</td>
</tr>
<tr>
<td><em>Chrysantellum americanum</em></td>
<td>greenish</td>
<td>pleasant scent</td>
<td>3,01</td>
</tr>
<tr>
<td><em>Tetrapleura tetraptera</em></td>
<td>brown</td>
<td>honey scent</td>
<td>3,84</td>
</tr>
</tbody>
</table>

The powders each have an odor but without a characteristic taste. These tests, although preliminary, sometimes make it possible to immediately recognize the plant drug, to check its degree of purity according to the presence or absence of foreign elements, moulds, etc. And possibly to detect adulteration or falsification [8, 14]. It is also possible to propose polyherbal teas by an association of 2 or 3 of these powders by exploiting its different indices.

The water loss of the samples ranged from 3.84% ± 0.11 to 7.39% ± 0.08 (Table 1). An excessively high percentage of water in a drug promotes a number of enzymatic reactions that can negatively affect the appearance of the drug, its organoleptic characteristics, and its therapeutic properties by degrading the active ingredients over time [14]. Thus, the content allowed in a medicinal product for a good conservation should not exceed 10% [21]. Thus, the moisture content of all our powders being less than 10%, this suggests that our herbal medicines could be stored for a long time with less risk of contamination and/or alteration of chemical principles.

3.2. Extraction and Chromatographic Fingerprint
The extracts a-d are obtained by depleting the different powders with solvents of increasing polarity. The extract e by aqueous decoction. The extraction yield obtained is given in the table below (Table 2). Extracts a (Hexanic extracts); Extracts b (dichloromethane extracts); Extracts c (Ethyl acetate extracts); Extracts d (butanolic extracts); Extracts e (aqueous decoction.)

<table>
<thead>
<tr>
<th>Extracts (Organic and Aqueous)</th>
<th>Extracts a</th>
<th>Extracts b</th>
<th>Extracts c</th>
<th>Extracts d</th>
<th>Extracts e</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cymbopogon citratus</em></td>
<td>1.51</td>
<td>2.27</td>
<td>3.43</td>
<td>4.64</td>
<td>23.375</td>
</tr>
<tr>
<td><em>Lippia chevalieri</em></td>
<td>2.57</td>
<td>2.50</td>
<td>3.90</td>
<td>4.88</td>
<td>16.96</td>
</tr>
<tr>
<td><em>Combretum micratum</em></td>
<td>1.67</td>
<td>2.05</td>
<td>4.25</td>
<td>5.86</td>
<td>16.34</td>
</tr>
<tr>
<td><em>Chrysantellum americanum</em></td>
<td>1.05</td>
<td>3.40</td>
<td>21.20</td>
<td>8.82</td>
<td>22.53</td>
</tr>
<tr>
<td><em>Tetrapleura tetraptera</em></td>
<td>10.00</td>
<td>2.93</td>
<td>2.61</td>
<td>5.86</td>
<td>39.46</td>
</tr>
</tbody>
</table>
The lowest yields are obtained with hexane and the highest with butanol and aqueous decoction. The yield of organic butanol and aqueous decoated extracts is related to the presence of polar compounds such as polyphenols. Regarding the developed chromatograms of the aqueous extract observed under the UV lamp at 366 nm, the plate was sprayed with a specific flavonoid reagent several distinct spots can be observed. The 5 chromatograms contain blue, yellow, brown, orange, green spots attesting the presence of flavonoids. The Tetrapleura tetraptera contains fewer spots and those found are blue in color. In all the samples we note the presence of phenolic compounds. A phytochemical screening would allow us to detect other phenolic compounds involved in the management of mental illness.

(1): Cymbopogon citratus; (2): Chrysanthellum americanum; (3): Combretum micratum (4): Lippia chevalieri; (5): Tetrapleura tetraptera

**Fig 1**: Fingerprint of aqueous extracts

### 3.3 Phytochemical Screening

The various extracts were analysed by TLC in order to highlight the chemical interest groups.

The results obtained are recorded in the following table (table 3).

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Terpenes</th>
<th>Phenolic compounds</th>
<th>Flavonoids</th>
<th>Acide phenolic</th>
<th>Saponins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cymbopogon citratus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lippia chevalieri</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Combretum micratum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chrysanthellum americanum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tetrapleura tetraptera</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

The phytochemical screening is carried out on TLC plates using specific reagents of some chemical groups. We use organic extracts obtained by fractionation of aqueous extracts. The screening of the different extracts revealed the presence of chemical interest groups in the selective extracts, namely terpenes, flavonoids, saponins and and phenolic acid. These results are consistent with the previous report on the phytochemistry of these different traditional drugs [9, 13, 23]. Preclinical studies have shown that certain compounds such as flavonoids, phenolic acids, saponins and terpenes, which have anxiolytic effects in a wide range of animal models, are involved in the treatment of mental disorders [22]. The presence of these compounds in all extracts justifies their sedative and anxiolytic properties. Phenolic compounds, particularly flavonoids, are found in all the extracts that were analyzed (figure 2). The content of these secondary metabolites could be used as an indicator for a quantitative evaluation of the bioactive compounds.
A quantitative phytochemical analysis of the aqueous extracts shows that the phenolic and flavonoid contents are high in all extracts. A comparison of the total phenolic and flavonoid contents based respectively on gallic acid and rutin as reference compounds indicates that the lowest content of phenolic compound is obtained with *Chrysantellum americanum* (167.65 ± 2.87 mg GAE/g extract) and the highest content is obtained with *Combretum micratum* extract (395.38 ± 7.05 mg GAE/g extract). The aqueous extract of *Combretum micratum* also gives the high content of total flavonoids (105.87 ± 0.05 mg Ru E/g extract). Thus, in the extract of *Combretum micratum* the content of total phenolics and total flavonoids is significantly higher than in the other extracts.

### 3.4 Phenolic Compounds determination

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Phenolic content</th>
<th>Flavonoids content</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cymbopogon citratus</em></td>
<td>186.53 ± 3.90</td>
<td>55.53 ± 0.05</td>
</tr>
<tr>
<td><em>Chrysantellum americanum</em></td>
<td>167.65 ± 2.87</td>
<td>60.78 ± 0.09</td>
</tr>
<tr>
<td><em>Combretum micratum</em></td>
<td>395.38 ± 7.05</td>
<td>105.87 ± 0.05</td>
</tr>
<tr>
<td><em>Lippia chevalieri</em></td>
<td>202.61 ± 2.50</td>
<td>38.13 ± 0.13</td>
</tr>
<tr>
<td><em>Tetrapleura tetraptera</em></td>
<td>191.11 ± 1.44</td>
<td>31.19 ± 0.05</td>
</tr>
</tbody>
</table>

### 3.5 Antiradical properties

The figure below shows the presence of light spots on a purple background. This shows that the extracts contain phytochemicals capable of trapping free radicals [20]. By superimposing the chromatographic profiles of the aqueous extract and those of the antiradical activity (figure 3), it is possible to establish the correspondence between the active areas and the nature of the compounds present. Indeed, the antiradical activity is more pronounced in extracts of *Combretum micratum*, *Lippia chevalieri*, *Tetrapleura tetraptera* compared to extracts of *Cymbopogon citratus*, *Chrysantellum americanum*, this could be explained by the high content of flavonoids in these decocted extracts. The antiradical activity of the different extracts shows that these extracts have antioxidant properties. The antioxidant activity of the compounds present in the extracts could also justify their use in the treatment of several diseases, including mental illnesses [9, 24].

![Fig 2: Highlighting of flavonoids in organic extracts (c and d).](image)

![Fig 3: Antiradical activity of aqueous extracts](image)

### 4. Conclusion

In this study, we analyzed the phytochemical profile of five plants involved in the treatment of mental disorders including *Cymbopogon citratus*, *Chrysantellum americanum*, *Combretum micratum*, *Lippia chevalieri* and *Tetrapleura tetraptera*. It appears from the analysis of the chemical profile of the different extracts that these plants contain bioactive compounds such as terpenes, flavonoids, phenolic acids and saponins. Phenolic compounds, particularly flavonoids, are present in high concentrations in the aqueous extracts. The antiradical activity on TLC plate is positive for all the extracts tested. This shows that these extracts also have antioxidant properties. Among the extracts studied, *Combretum micratum* showed the highest content of total phenolic and total flavonoids. The extract of this plant is also the most positive in the antiradical test. A combination of these plants containing *Combretum micratum* could prove beneficial and more effective than any of the five plants used separately.
5. References


