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The effect of blending of extracts of Sudanese *Adansonia digitata* and *Tamarindus indica* on their antioxidant, anti-inflammatory and antimicrobial activities

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*Adansonia digitata* (Bombacaceae) and *Tamarindus indica* (Fabaceae) are the most popular herbal products in Sudan; they are used as food ingredients and in traditional treatments of various diseases. The aim of this study is to investigate phytochemical contents, antioxidant, anti-inflammatory and antimicrobial activities of plants fruit, leaf and bark ethanolic extracts. The phytochemical screening of different extracts revealed the presence of alkaloids, flavonoids, sterols/triterpens, tannins, saponins, coumarins, glycosides, reducing sugar, lignin and carbohydrates. The results indicate that all the extracts have reducing power DPPH radical scavenging abilities. The highest antioxidant activity showed in *A. digitata* parts. The fruit extracts of both plants showed the highest antioxidant activity (84.07 and 83.98% for *A. digitata* and *T. indica*, respectively). The in vitro anti-inflammatory effects have been studied by human albumin denaturation, and both plant extracts showed remarkable activity. Leaf extracts showed highest anti-inflammatory activity (76.71, 62 and 82.71% for *A. digitata*, *T. indica* and mixture of both respectively). The results of antimicrobial activity showed the effectiveness of mixture extracts against tested standard pathogens. Fruit mixtures showed the highest activity against *B. subtilis* (19 mm), *S. aureus* (35 mm) and *S. typhi* (21 mm), while the mixture of bark extracts showed the highest activity against *E. coli* (19 mm). It is noteworthy that higher antioxidant, anti-inflammatory and antimicrobial activities have been observed by blends in the ratio 1:1 of fruit, leaf and bark extracts of both plants.

**Key words:** *Adansonia digitata*, *Tamarindus indica*, antioxidant, anti-inflammatory, antimicrobial, combination.

**INTRODUCTION**

Sudan traditional medicine is characterized by a unique combination of Islamic, Arabic, and African cultures. In
poor communities, traditional medicine has remained as the most reasonable source of treatment of several diseases and microbial infections. Although the traditional medicine is accepted in Sudan, to date there is no updated review available which focuses on most effective and frequently used Sudanese medicinal plants (Karar and Kuhnert, 2017).

Adansonia digitata (Bombacaceae) known as Baobab is an important plant used in Sudanese traditional medicine; it is widespread throughout the hot, drier regions of tropical Africa. It extends from northern Transvaal and Namibia to Ethiopia and Sudan. In Sudan, the baobab is most frequently found on sandy soils and by seasonal streams Khors in short grass savannas. It forms belts in Central Sudan, Kordofan, Darfur and Blue Nile (Dabora, 2016). The Baobab fruit pulp is an important foodstuff used as a drink, a sauce for food and as a fermenting agent in local brewing (Gebauer et al., 2002). Different parts of the plant are used to treat many diseases. The alkaloid ‘adansoniin’ in the bark is thought to be the active principle for treatment of malaria and other fevers (De Caluwé et al., 2010). The plant is: Antioxidizing agent including; polyphenolic compounds, vitamins E and C, cardiovascular diseases, cancer and aging related disorders (De Caluwé et al., 2010); antiviral activity against Herpes simplex, Sindbis and Polio (Anani et al., 2000); anti-inflammatory and antipyretic activity (Kaboré et al., 2011); anti-microbial activity and anti-trypanosoma activity (Varudharaj et al., 2015). Tamarindus indica (Fabaceae) grows wild in Africa in locales as diverse as Sudan, Cameroon, Nigeria, and Tanzania (Havinga et al., 2010). Tamarind fruit pulp is used for seasoning as a food component to flavour confections, curries and sauces, and is a main component in juices and certain beverages. Tamarind fruit pulp is eaten fresh and often made into a juice, infusion or brine, and can also be processed into jam and sweets (Hassan, 2014). The plant is widely used in African traditional medicine for treatment of many diseases such as fever, dysentery, jaundice, gonococci and gastrointestinal disorders (Lawal et al., 2010). Phytochemical investigation carried out revealed the presence of many active constituents, such as phenolic compounds, cardiac glycosides, L-(-)-malic acid, tartaric acid, the mucilage and pectin, arabinose, xylose, galactose, glucose, and uronic acid. The ethanolic extracts showed the presence of fatty acids and various essential elements like arsenic, calcium, cadmium, copper, iron, sodium, manganese, magnesium, potassium, phosphorus, lead, and zinc (Bhadoraya et al., 2011). Many studies have been performed on skin, eye and respiratory tract irritation mediated by complex mixtures, but only few studies allows a quantitative evaluation of the modulating effects of the combination of single chemicals. Further studies on the modulating effects of the combination of chemicals concerning skin, eye and respiratory tract irritation will be required in order to evaluate the possibilities for synergistic or antagonistic effects being mediated by mixtures of chemicals (Doty et al., 2004). The present study reports the effect of blending equal amounts of A. digitata (Bombacaceae) and T. indica (Fabaceae) fruit, leaf and bark 96% ethanolic extract on their antioxidant, anti-inflammatory and antimicrobial activities.

MATERIALS AND METHODS

Preparation of plants extracts

The fruit, leaves and bark of A. digitata and T. indica were collected from Abu karshola, West Kordofan State, Sudan, in December, 2017. The specimens were deposited in the herbarium of medicinal and aromatic plants institute, Khartoum, Sudan. The fresh samples were cleaned, air dried and ground to powder using a pestle and mortar. Fifty grams of each powdered sample was extracted with 96% ethanol at room temperature for 72 h, filtered through Whatman number 4 filter paper and concentrated in a rotatory evaporator under reduced pressure.

Phytochemical analysis

Qualitative preliminary phytochemical analysis was performed with different chemical reagents to detect the nature of phytoconstituents and their presence in the samples. The presence of sterols/terpenes, flavonoids, tannins, alkaloids, saponins and coumarins was evaluated by standard qualitative methods (Hameed, 2012).

Antioxidant activity

The DPPH radical scavenging was determined with some modification (Shimada et al., 1992). In 96-wells plate, the test samples were allowed to react with 2,2-Di (4-tert-octylphenyl)-1-picryl-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 UV spectrophotometer double beam. Ascorbic acid was used as standard. The ability to scavenge of the DPPH radical was calculated using the following equation:

\[
\text{DPPH scavenging activity } (\%) = \left( \frac{A_0 - A_1}{A_0} \right) \times 100
\]

Where A0 is the absorbance of the control and A1 is the absorbance of the sample.

*Corresponding author. E-mail: Ayatwarag@yahoo.com.

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Table 1. Yield percentage of different parts extracts of *A. digitata* and *T. indica*.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Yield (%)</th>
<th><em>A. digitata</em></th>
<th><em>T. indica</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit</td>
<td>4.64</td>
<td>22.84</td>
<td></td>
</tr>
<tr>
<td>Leave</td>
<td>14.7</td>
<td>14.5</td>
<td></td>
</tr>
<tr>
<td>Bark</td>
<td>1.9</td>
<td>5.08</td>
<td></td>
</tr>
</tbody>
</table>

Anti-inflammatory activity

**Inhibition of albumin denaturation**

Inhibition of protein denaturation was evaluated by the method of Mizushima and Kobayashi with slight modification: 500 μL of 1% bovine serum albumin was added to 100 μL of plant extract with different concentrations. This mixture was kept at room temperature for 10 min, followed by heating at 51°C for 20 min. The absorbance was recorded at 660 nm. (Chandra et al., 2012). Percent inhibition for protein denaturation was calculated using:

\[
\text{% Inhibition} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100
\]

Where A0 is the absorbance of the control and A1 is the absorbance of the sample.

Antimicrobial activity

**Test microorganisms**

Bacteria organisms used were *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*. The fungal organisms used were *Candida albicans* and *Aspergillus niger*. Standard strains of microorganism used in this study were obtained from medicinal and aromatic plants research institute.

**Antibacterial assay**

The disc-diffusion assay with some modifications was employed to investigate the inhibition of bacterial growth by plants extract (Kil et al., 2009). Extract solution (20 mg/ml) was prepared by diluting with dimethyl sulfoxide (DMSO) 30%. Base plates were prepared by pouring 10 ml Mueller-Hinton (MH) agar into sterile Petri dishes. About 0.1 ml of the standardized bacterial stock suspension 10^8 to 10^9 C.F.U/ ml were streaked on Mueller Hinton agar medium plates using sterile cotton swab. Sterilized filter paper disc (6 mm diameter) were soaked in the prepared extracts, and then were placed on the surface of the test bacteria plates. The plates were incubated for 24 h and the diameters of the inhibition zones were measured.

**Antifungal assay**

The same method described for bacteria was employed to assess antifungal activity, Sabouraud Dextrose Agar was used. The inoculated medium was incubated at 25°C for two days for the *C. albicans* and three days for *A. niger*.

RESULTS AND DISCUSSION

**Extraction of the plants**

Among the extracts obtained using 96% ethanol, *T. indica* fruits gave the highest yield of 22.84% as shown in Table 1, leaf extracts of both plants gave the same yield of 14%, while the bark extract of *A. digitata* gave the lowest yield of 1.9%.

**Phytochemical screening of *T. indica* and *A. digitata***

The extracts of *T. indica* contained some secondary metabolites; most of them present in leaves and bark including alkaloids, flavonoids, glycosides, terpenoids, coumarins, tannins, saponins, reducing sugars and carbohydrates. Antibacterial activity of Tamarind leaf extract was attributed to the presence of flavonoids, alkaloids, tannins, cyanogenic glycosides and anthraquinones. It is known that these phytochemicals and some other aromatic secondary metabolites may serve as natural agents that protect plants against microbial pathogens and insect predators. Phytochemicals may act like antioxidants to facilitate, protect and regenerate essential nutrients and/or work to deactivate cancer-causing substances (Gomathi et al., 2017). The results of the phytochemical screening showed that *A. digitata* fruit, leaf and bark extracts were rich in alkaloids, flavonoides, sterols, triterpines, tannins, saponins, coumarins, glycosides, reducing sugar and carbohydrates in all parts, but anthraquinones were not detected (Table 2). Many of these are known to provide protection against insects' attacks and plant diseases. The results obtained seems to justify the use of leaf of *A. digitata* in African dishes as it contained appreciable amount of some important compounds such as phenols, saponins, flavonoids, alkaloids. It is also possible that these plant species could have allelopathy effect on other organisms in their ecotype since these bioactive substances are responsible for such actions. Cardiac glycoside detected in this plant indicated that the plant could be a good source for birds and insect repellants (Lock et al., 2016).
Table 2. Phytochemical screening of *T. indica* and *A. digitata* parts extracts.

<table>
<thead>
<tr>
<th>Test</th>
<th>Specific test</th>
<th>Fruit</th>
<th>Leave</th>
<th>Bark</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>T. indica</em></td>
<td><em>A. digitata</em></td>
<td><em>T. indica</em></td>
<td><em>A. digitata</em></td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Wagner’s</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td></td>
<td>Mayer’s</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>Dragendroff’s</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>FeCl₃</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td></td>
<td>Lead acetate</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Sterols</td>
<td>Salkowski</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>Lebermann</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Triterpines</td>
<td>Salkowski</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td></td>
<td>Leberman</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Tannins</td>
<td>Gelatin</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>Lead acetate</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Saponins</td>
<td>Foam test</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>UV lamp</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Keller kiliani</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>Kedd’s</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>Fehlings</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Lignin</td>
<td>Labat test</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>Molich</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
</tbody>
</table>

DPPH radical scavenging activity

![DPPH Radical Scavenging Activity](image)

**Figure 1.** Antioxidant activity of *T. indica* and *A. digitata*.

**Antioxidant activity**

**DPPH radical scavenging activity**

The *in vitro* antioxidant activity of fruit, leaf and bark ethanolic extracts of *T. indica* and *A. digitata* was evaluated using DPPH assay. Results are shown in Figure 1. The highest result of antioxidant activity by DPPH scavenging assay in fruit extract in Tamarind (83.98%) followed by Baobab (83.98%). The result is very high compared with ascorbic acid (93.5%) as antioxidant standard. The leaves of Tamarind showed
higher activity (61.66%) than Baobab (15.69 %), whereas the bark showed 51.48 and 66.90% in Tamarind and Baobab respectively. Several reports indicated that the antioxidant potential of medicinal plants may be related to the concentration of their phenolic compounds which include phenolic acids, flavonoids, anthocyanins and tannins (Djeridane et al., 2006; Wong et al., 2006). The health benefit of fruits are mainly attributed to phenolic compounds and vitamins, which enhance their antioxidant, anticancer, anti-mutagenic, antimicrobial, anti-inflammatory and neuroprotective properties. Bio-guided fractionation of extracts might promote the development of alternative therapeutic compounds for the prevention and treatment of various diseases and disorders.

**Anti-inflammatory activity**

Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. The results showed both ethanolic extracts leaves parts represent the highest anti-inflammatory activity even in mixture form, followed by fruits, then bark, while in individual manner bark extracts represent higher activity than fruits as shown in Table 3. The results showed a concentration-dependent inhibition of protein (albumin) denaturation by *A. digitata* and *T. indica* extracts. *T. indica* extracts expressed a higher activity compared to *A. digitata*. Previous study by Osman and Idrees (2017) reported that both *A. digitata* and *T. indica* extracts exerted marked anti-inflammatory properties.

**Antimicrobial activity**

**Antibacterial activity**

Diseases such as bacterial, fungal and infectious parasitic are mainly controlled by chemotherapeutics and antibiotics in aquaculture. Nevertheless, the uses of antibiotics and chemotherapy have been strongly criticized as they created problems with drug resistance bacteria (Harikrishnan et al., 2011). All tested extracts showed various degrees of biological activity on the tested pathogens. All plants parts combinations showed remarkable activity against tested bacteria and fruit mixtures showed the highest activity against *B. subtilis* (19 mm), *S. aureus* (35 mm) and *S. typhi* (21 mm), while the mixture of bark extracts showed high activity against *E. coli* (19 mm). The results are presented in Table 4. Plant extracts are an important part in agroecology, as they benefit the environment in combating pathogenic organisms, without resorting to synthetic chemicals. (Rivera et al., 2014). Previous study showed that the significant differences observed in antibacterial activities

### Table 3. Inhibition of albumin denaturation.

<table>
<thead>
<tr>
<th>Sample</th>
<th><em>T. indica</em> (%) inhibition</th>
<th><em>A. digitata</em> (%) inhibition</th>
<th>Mixture (%) inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit</td>
<td>55.85</td>
<td>50</td>
<td>73.42</td>
</tr>
<tr>
<td>Leave</td>
<td>76.714</td>
<td>62</td>
<td>82.71</td>
</tr>
<tr>
<td>Bark</td>
<td>57.642</td>
<td>54.92</td>
<td>59.14</td>
</tr>
</tbody>
</table>

### Table 4. Antimicrobial activity of plant extracts against bacteria pathogens.

<table>
<thead>
<tr>
<th>Part of plant</th>
<th>Sample</th>
<th><em>Bacteria strain</em> (M.D.I.Z)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>B. subtilis</em></td>
</tr>
<tr>
<td>Fruit</td>
<td><em>A. digitata</em></td>
<td>12±0.00</td>
</tr>
<tr>
<td></td>
<td><em>T. indica</em></td>
<td>12±1.41</td>
</tr>
<tr>
<td></td>
<td>Mixure</td>
<td>19±0.00</td>
</tr>
<tr>
<td>Leave</td>
<td><em>A. digitata</em></td>
<td>11±2.12</td>
</tr>
<tr>
<td></td>
<td><em>T. indica</em></td>
<td>12±0.70</td>
</tr>
<tr>
<td></td>
<td>Mixure</td>
<td>18±0.00</td>
</tr>
<tr>
<td>Bark</td>
<td><em>A. digitata</em></td>
<td>11±0.00</td>
</tr>
<tr>
<td></td>
<td><em>T. indica</em></td>
<td>12±0.70</td>
</tr>
<tr>
<td></td>
<td>Mixure</td>
<td>16±0.00</td>
</tr>
</tbody>
</table>

*M. D. I. Z.*, Mean diameter of growth inhibition zone in mm.
suggest that extract mixtures affect in a different way each of the tested food-borne pathogen bacteria; while differences among extract mixtures suggest that at least one extract mixture affect in a different way the bacterial growth. *A. digitata* leaves contain active ingredients against *S. aureus, B. subtilis, P. aeruginosa, S. typhi, C. albicans, A. niger, and P. rotatum* (Abiona et al., 2015).

### Antifungal activity

All extracts and blends of the two plants were tested for their antifungal activity and the results are shown in Table 5. The inhibitory percentage of extracts and their combinations against *C. albicans* ranged from 13-32 mm and 13-36 mm against *A. niger*. The blends of all plant extracts of the same part showed higher activity than original extracts of the same plant.

Remarkable synergistic effect of plant extracts was observed because the plants have the same pharmacological activity, and the active compounds present in each extract act together to targeted receptors, therefore producing higher effect than using any plant extract alone. From other point of view, some of the plants compounds may not have pharmacological activity but act as a potentiated agents to improve the activity of the compounds found in the other plant extract with pharmacological activity (Atanasov et al., 2015).

### Conclusion

In this study different part of *A. digitata* and *T. indica* were used. The plants are rich sources of chemical and bioactive compounds including therapeutic and dietary constituents. The present study suggests that blending of the plants parts of *A. digitata* and *T. indica* could be a potential source of natural antioxidant and anti-inflammatory properties that could have great importance in the inhibition of inflammation as well as against bacterial and fungal infections. The findings of this study suggest that the tested plants and their combinations can be developed as effective herbal phyto-pharmaceutical drugs for treatment and nutrition. Based on the research outcomes we recommend conducting more experiments on the combination such as anticancer activity, bacterial and fungal infections and virus infections.

### CONFLICT OF INTERESTS

The authors have not declared any conflict of Interests.

### ACKNOWLEDGEMENTS

The present work is the second part of devoted interdepartmental on-going research in the Faculty of Pharmacy, UMST. The authors are grateful to the dean of the Faculty of Pharmacy Prof. Abdelazim Elshaikh and to the administration of the UMST for their support.

### REFERENCES


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**Table 5. Antifungal activity of parts extracts against *C. albicans* and *A. niger.***

<table>
<thead>
<tr>
<th>Part of plant</th>
<th>Sample</th>
<th>Fungi (M.D.I.Z)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>C. albicans</em></td>
<td><em>A. niger</em></td>
</tr>
<tr>
<td>Fruit</td>
<td><em>A. digitata</em></td>
<td>15±1.41</td>
</tr>
<tr>
<td></td>
<td><em>T. indica</em></td>
<td>18±0.70</td>
</tr>
<tr>
<td></td>
<td>Mixure (1:1)</td>
<td>32±0.70</td>
</tr>
<tr>
<td>Leaves</td>
<td><em>A. digitata</em></td>
<td>16±0.70</td>
</tr>
<tr>
<td></td>
<td><em>T. indica</em></td>
<td>13±1.41</td>
</tr>
<tr>
<td></td>
<td>Mixure (1:1)</td>
<td>28±1.41</td>
</tr>
<tr>
<td>Bark</td>
<td><em>A. digitata</em></td>
<td>20±0.00</td>
</tr>
<tr>
<td></td>
<td><em>T. indica</em></td>
<td>15±1.41</td>
</tr>
<tr>
<td></td>
<td>Mixure (1:1)</td>
<td>30±0.00</td>
</tr>
</tbody>
</table>

*M. D. I. Z., Mean diameter of growth inhibition zone in mm.*


Dabora SA (2016). Assessment of the effect of addition of Baobab (Adansonia digitata L.) fruit pulp on properties of camel milk yoghurt, Sudan University of Science and Technology.


Hameed M (2012). Pharmacognostic Study Of Five Medicinal Plants Of Family Solanaceae From District Peshawar, Pakistan, University of Peshawar.


Full Length Research Paper

Immunomodulatory activities of polysaccharides isolated from plants used as antimalarial in Mali

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Medicinal plants used against malaria in Mali have previously been tested for their antiplasmodial activities using their organic solvent and water extracts. As the healers mainly use the water extracts for their treatments of malaria-patients, our aim was to study the water-soluble components from Malian plants used for treatment of malaria. *Argemone mexicana* (aerial parts), *Sarcocephalus latifolius* (root bark), *Vitex doniana* (leaves), and Malarial-5® (an improved traditional medicine (ITM) in tea) were the objects of our studies. Water extracts of these plants contained primarily polysaccharides. Due to this, the studies focused on the determination of the monosaccharide composition of the polymers present as well as assessing the immunomodulatory properties of the polysaccharide fractions isolated from these plants. Each plant material was extracted sequentially with dichloromethane, 80% ethanol and water at 100°C. The polysaccharides were obtained using gel filtration of the aqueous extracts and their monosaccharide compositions were determined using gas chromatography. Immunomodulatory effects were assessed using the complement fixation test and macrophage stimulation. All aqueous extracts from the four samples contained polysaccharides. The monosaccharide compositions vary between the plants. Arabinose, rhamnose, galactose, glucose and galacturonic acid were present in all samples, glucose being the main monomer. These polysaccharides showed complement fixing activity and induced nitrite oxide release from macrophages in a dose dependent manner. The polysaccharide fractions of *A. mexicana* (Am1) and *V. doniana* (Vd1) showed the most potent activities. These two fractions had an ICH50 of 2.4 and 6.3 μg/mL respectively in the complement fixation assay. The same two fractions induced a dose dependent release of nitrite oxide from macrophages. The results demonstrated that antimalarial plants contain polysaccharides with immunomodulatory properties. This preliminary work constitutes a new approach of antimalarial studies.

Key words: Polysaccharides, immunomodulatory effects, antimalarial plants, Mali.

INTRODUCTION

In 2017, World Health Organization (WHO) estimated 219 million cases of malaria in 90 countries with 435,000 deaths worldwide. The African region registered 92% of the malaria cases and 93% of the deaths (WHO, 2017).
This infectious disease caused by parasites is transmitted to people through the bites of infected female anopheles mosquitoes (WHO, 2017). *Plasmodium falciparum* is mainly responsible for the enormous deaths (99%), rarely caused by other *Plasmodium* species (Belachew, 2018). Malaria is the primary cause of morbidity and mortality in Mali, particularly among children under the age of five (Anonymous, 2018). The entire population of Mali is at risk for malaria. The disease is endemic in the central and southern regions where more than 90 percent of the population lives, and it is also epidemic in the north (Anonymous, 2018).

In Mali, the current health system is decentralized, and is composed of three levels, which involves an integrated community case management package at the community level (Anonymous, 2018). Most of the population is still using traditional medicines for their primary health care. Plants have been used to treat malaria for thousands of years and are the source of the two main groups of modern antimalarial drugs (quinine and artemisinin derivatives) according to Willcox and Bodeker, (2004). RTS,S/AS01 (RTS,S) also known as Mosquirix® is an injectable vaccine that offers protection against malaria in young children, but it is unavailable for the population where malaria is endemic (Anonymous, 2018). The problems of increasing levels of artemisinin-resistant parasites encourage researchers for finding a new source of anti-parasitic drugs. Since developing countries have difficulties in affording and accessing effective antimalarial drugs, traditional medicines could be an important and sustainable source of treatment (Willcox and Bodeker, 2004). Therefore the exigent need of effective molecules remains a huge challenge for scientists. Most of the recent investigations on antimalarial plants have been focused on organic solvent extracts. But in Mali, the department of traditional medicine (DMT) in order to supersede its first Malian improved traditional medicine for malaria (Malarial-5®) has demonstrated the efficacy and safety of aqueous extracts through preclinical and clinical studies including *Sumafura Tiemoko Bengaly* herbal tea based on *Argemone mexicana* that came out from a retrospective treatment-outcome study (Diallo et al., 2007; Willcox et al., 2007; Sanogo et al., 2008; Graz et al., 2010; Willcox et al., 2011). The phytochemical analysis and biological activities on *Sumafura Tiemoko Bengaly* an herbal tea led to formulate syrups for an efficient utilisation and its standardization (Sanogo et al., 2012, 2014). This new antimalarial phytomedicine made by the department of traditional medicine of Mali retrieves its name from one traditional healer, *Tiemoko Bengaly*, who has participated in its development (Willcox, 2011). This author reported that several alkaloids including berberine, protopine, and allocryptopine from *A. mexicana* exhibited *in vitro* antimalarial effect while animal studies suggest that the crude aqueous extract is not effective against *Plasmodium berghei*, and berberine also is not well absorbed orally (Willcox, 2011). Thus, some investigations were underway to identify which compounds are active in humans. Most of traditional healers in Mali are thinking that plants mainly should be taken as water extract. Therefore a new approach is urgently needed to find a product for prevention and treatment of malaria. Although parasites have their own ways to develop resistance against drugs, the immune system has naturally evolved to arm the host against pathogens, including parasites. Both innate and adaptive immune responses selectively recognize pathogens and help the host to get rid of many of them at first sight (Coban and Yamamoto, 2018). Due to this, our theory is that the healing effect observed with patients using aqueous plant extracts could partly be due to their stimulating activity of the immune system.

In Malian traditional medicine, water decoction is the most popular mode of preparation of plants remedies and polysaccharides isolated from those crude water extracts have shown effects related to the immune system through various *in vitro* and *in vivo* tests (Paulsen and Barsett, 2005). Diallo and coworkers showed that the pectic polysaccharides isolated from the leaves of *Trichilia emetica* (Meliaceae), a plant used in traditional medicine in Mali, activated the complement system and induced the proliferation of T and B-lymphocytes (Diallo et al., 2003). *Biophytum peterianum*, traditionally used in Mali for wound healing contains polysaccharides with complement fixing activity (Inngjerdingen et al., 2006; Inngjerdingen et al., 2008; Grønhaug et al., 2011). *Opilia cellidifolia* used traditionally against skin diseases and malaria is also known as appetizer plant in Mali. Polysaccharide fractions from that species exhibited complement fixation and macrophage stimulation activities (Diallo et al., 2003; Togola et al., 2008; Šutovská et al., 2009). Investigations on the roots of *Vernonia kotschyanana* used to produce Gastroesdal, an improved traditional medicine in Mali, revealed that its polysaccharides possessed a complement fixation activity (Nergard et al., 2005; Inngjerdingen et al., 2012). Recently, two other Malian medicinal plants, *Parkia biglobosa* and *Terminalia macroptera* were reported to contain polysaccharides having complement fixing and macrophage stimulating effects (Zou et al., 2014a, b). Often, pectic extracts prepared using hot water, were found to be active on the complement system. Abouge-Angone et al. (2011) reported that plant water soluble...
compounds like pectic and hemicellulosic polysaccharides have immunomodulatory and mitogenic (proliferation of B-lymphocytes) properties. Plant polysaccharides can directly activate the immune function of macrophages, T or B lymphocytes, natural killer cells, and complement (Yu et al., 2017). Macrophages stimulated with lipopolysaccharides (LPS) or an immunomodulatory compound produce large amounts of free radicals such as nitric oxide (NO), which effectively suppressed the blood stage of the malarial parasite (Awasthi et al., 2003). Thus, the present study aimed to investigate the components and the immunomodulatory effects of polysaccharides from antimalarial plants used in Mali.

MATERIALS AND METHODS

Plant material

Three plants and one improved traditional medicine used frequently in Mali against malaria without prior knowledge on their immunomodulatory properties were selected for the present study. The plant materials were A. mexicana L., Papaveraceae (aerial parts), Sarcopodium latifolius (Sm.) E.A. Bruce, Rubiaceae (root bark), Vitex doniana Sweet, Lamiaceae (leaves), and Malaria-56 (improved traditional medicine containing Senna occidentalis (L.) Link. (syn. Cassia occidentalis L.) (leaves), Lippia chevalieri Moldenke (leaves) and Acmella oleracea (L.) R.K.Jansen. (syn. Spilanthes oleracea L.) (flowers) presented as herbal tea. A. mexicana, S. latifolius and V. doniana, were bought at the market of Medina in Bamako, Mali, in 2012, identified by Professor Drissa Diallo, Department of Traditional Medicine, Bamako, Mali, and voucher specimen were deposited at the herbarium of the DMT (Voucher No 2948 / DMT, 2198 / DMT, 2008 / DMT respectively). The Plant List website (www.theplantlist.org) was accessed in February 2019 for correct Latin names of the plants. The plant materials were air dried at room temperature and pulverized into fine powder by a mechanical grinder. The tea form of Malaria-56, which is an improved traditional medicine of DMT, was provided by this institution. The powders and the herbal tea were used for the extraction.

Method of extraction

The powdered leaves, root bark, aerial parts and the formulated herbal tea (50 g of each) were extracted with dichloromethane using the Soxhlet system, followed by maceration in 80% ethanol in order to remove lipophilic compounds and colored materials. The residues were then extracted with water at 100 °C for 1 h, filtered through glass fiber filter, and concentrated at 40°C under vacuum with an evaporator. The concentrated solutions were frozen to give the crude water extracts called Vd, Sl, Am and Ma respectively for V. doniana, S. latifolius, A. mexicana and Malaria-56.

Fractionation of polysaccharides by chromatography on Biogel P6

The aqueous extracts (Vd, Sl, Am and Ma) were dissolved in distilled water, centrifuged, filtered through a Millipore filter (5 µm) and gel filtered on a Biogel P6 column (5 cm × 60 cm) using distilled water as the mobile phase. The fractions, from high to low molecular weights, were identified based on their elution profiles as tested by the phenol sulphuric acid method (Dubois et al., 1956). Three fractions (1, 2 and 3) of Vd, Sl and Am; and two fractions (1 and 2) of Ma contained high molecular weight material. Each fraction was pooled, concentrated and freeze-dried to give polysaccharide fractions. The samples retained for further studies were called Vd1, Vd2, Vd3, Sl1, Sl2, Sl3, Am1, Am2, Am3, Ma1 and Ma2.

Determination of monosaccharide composition of the fractions

One milligram of the lyophilized polysaccharide of each sample was subjected to methanolysis for 24 h (80°C) using water free 3 M HCl in MeOH (Sigma–Aldrich) (Chambers and Clamp, 1971). Hundred microliters of mannitol (1 mg/mL) were added as an internal standard. After 24 h reaction time, the reagents were removed with nitrogen and the methyl-glycosides dried in vacuum over P2O5 for 1 h prior to their conversion into the corresponding trimethyl silyl ethers (TMS-derivates). The samples were analyzed by capillary gas chromatography (30 m × 0.32 mm, J and W Scientific Inc.) on a Carlo Erba 6000 Vega Series 2 gas chromatograph with an ICU 600 programmer (Chambers and Clamp, 1971; Barsett et al., 1992). The injector temperature was 250°C, the detector temperature 300°C and the column temperature was 140°C when injected, then increased with 1°C/min to 170°C, followed by 6°C/min to 256°C and then 3°C/min to 300°C. Helium was used as carrier gas with a flow rate adjusted to a retention time of 33 min for the internal standard. Based on standards for all the monomers present, the monosaccharides were identified and quantified.

Immunomodulatory activities

The complement fixation test

The complement fixation test is based on inhibition of haemolysis of antibody sensitized sheep red blood cells (SRBC) by complement from human sera as described by Michaelisen et al. (2000). Sheep erythrocytes were washed twice with 9 mg/mL NaCl and once with veronal buffer (VB) pH 7.2 containing 2 mg/mL bovine serum albumin (BSA) and 0.02 % sodium azid (VB/BSA) and sensitized with rabbit anti-sheep erythrocyte antibodies (Viron anticomceptor 9202, Ruschlikon, Switzerland). After incubation at 37°C for 30 min, the cells were washed as described above, and 1% cell suspension in veronal buffer was prepared. The serum was diluted with VB/BSA to a concentration giving about 50% haemolysis. Samples were dissolved in VB/BSA (500 µg/mL) and a 4-fold dilution made. Sample dilutions (50 µL) and serum dilution (50 µL) were added in duplicates into the wells of a round bottom microtiter plate and incubated on a shaker at 37°C. After 30 min, the sensitized sheep erythrocytes (50 µL) were added and the microtiter plate incubated again as earlier described. After centrifugation at 1000 x g for 5 min, 100 µL of the supernatants were transferred to a flat bottom microtiter plate and the absorbance was read at 405 nm using a microplate reader. Hundred percent of lysis were obtained with distilled water and sensitized sheep erythrocytes. VB/BSA, serum and sensitized sheep erythrocytes were the control of the medium, and the pectin fraction, BP11 from the leaves of B. petersianum was used as positive control. The prevention or inhibition of lysis induced by the test sample was calculated by the following formula:

$$\left[ \frac{(A_{control} - A_{test})}{A_{control}} \right] \times 100\%$$

Acontrol is the absorbance of control and Atest is the absorbance of test sample.

From these data, a dose-response curve was constructed and the concentration of test sample giving 50% inhibition of haemolysis
was calculated. A low ICH50 value means a high complement fixing activity. This biological test system can have some day to day variations, and thus, the ratio ICH50-BPII / ICH50- sample was calculated. A high ratio means high complement fixing activity.

**Analysis of nitric oxide (NO) production**

Nitric oxide (NO) released by activated macrophages is broken down to nitrite (NO2−) in the medium, which can be measured in a colorimetric assay using the Griess reagents. The mouse macrophage cell line Raw 264.7 was cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, antibiotics, L-glutamine, and 5×10⁻⁴ M 2-mercaptoethanol, and split every second day. Macrophages at a density of 5×10⁵ cells/mL were seeded into 96-well flat-bottomed plates, and stimulated for 22 h in duplicates with increasing concentrations (1,10,100 μg/mL) of samples (Vd1, Vd2, Sl1, Sl2, Am1, Am2 and Ma1 selected from their effect in the complement fixing test), LPS (from *P. aeruginosa* 10, Sigma–Aldrich) and the pectic polysaccharide Oc50A1.I.A. The polysaccharide fraction *O.celtidifolia* (Grenhaug et al., 2010) as positive controls, or medium alone. Nitrite was then determined in cell-free supernatants. The supernatant (50 μL) was mixed with an equal volume of Griess reagent A (1% [w/v] sulfanilamide in 5% [v/v] phosphoric acid) and incubated at room temperature in the dark for 10 min. After addition of 50 μL 0.1% (w/v) N-(1-naphthyl) ethylenediamine dihydrochloride in water (Griess reagent B) the absorbance was read at 540 nm. A dilution series of NaNO₂ was used as a standard reference curve. The experiment was repeated two times, and the results shown are expressed as the mean ± SEM.

**RESULTS AND DISCUSSION**

**Polysaccharide fractions**

Eleven polysaccharide fractions Vd1, Vd2, Vd3, Sl1, Sl2, Sl3, Am1, Am2, Am3, Ma1 and Ma2 (Vd, Sl, Am and Ma indicate the fractions respectively for *V. doniana*, *S. latifolia*, *A. mexicana* and *Malarial-5*) were extracted as presented in Table 1. The fraction Vd3 presented the highest yield while Ma1 showed the lowest amount. Based on literature, this is the first time to extract polysaccharides from these plant species. These fractions with undefined different molecular weights were the objects for further studies. These fractions could contain both neutral and acidic polysaccharides as they were not separated. This was not done as it was important to have all the water soluble materials present in the fractions that were tested for bioactivities, as those were as close as possible to those extracts prepared by the traditional healers.

**Carbohydrate composition of the fractions**

The monosaccharide compositions of the eleven fractions were determined and the results are presented in Table 2. The monosaccharide composition is typical for pectins like starch. Glucose was present in high amounts in all fractions. The other monosaccharides, galacturonic acid (Gal A), rhamnose (Rha), arabinose (Ara) and galactose (Gal), are all recognized as typical constituents in pectic polysaccharides (Inngjerdingen et al., 2012). The polysaccharides were mainly Gal A (39.9%), Ara (14.7%) and Gal (13.5%) for Vd1, while Vd2 had Gal (15.1%) as the major. The polysaccharide fraction Sl1 had Gal (14.8%) and Ara (14.4%) as the major pectic monomers, Am1 had Gal (39.8%) and Ara (22.3%), while Am2 was rich in GalA (12.0%) and Rha (10.3%). The major pectic monosaccharides of the polysaccharide fraction Ma1 were Gal A (23.8%), Ara (23.7%) and Gal (22.5%). The presence of these monosaccharides proved polysaccharide existence in the investigated antimalarial plants and also that they could be of the pectic type polysaccharides (Inngjerdingen et al., 2012). The high content of glucose after methanolysis could be related to starch (Inngjerdingen et al., 2012). In the presence of fair amount of xylose in some samples could explain that some of the glucose also could be due to xyloglucan. All fractions contained arabinose and galactose, indicating the presence of arabinogalactans, polymers which are commonly present in pectin as side chains on the main core. In addition, the presence of galacturonic acid and rhamnose could indicate that the polymers may contain a main core consisting of a rhamnogalacturonan (indicative of RG I) linked with longer chains of homogalacturonan as noted by Braünlich et al. (2018).

**Immunomodulatory activities**

**Complement fixation activity**

The ICH50 values of the polysaccharide fractions and the ratio ICH50 BPII/ICH50 sample are given in Table 3 and Figure 1 respectively. The polysaccharide fraction (Am1) was the fraction with the highest activity, more potent than all the other polysaccharide fractions and approximately 8.3 times stronger than the standard polysaccharide (BPII a pure pectic AGII type polysaccharide isolated from *B. petersianum*). It is interesting to also note that the polysaccharide fraction (Vd1) had a relative high activity, approximately 3 times stronger than the standard polysaccharide (BPII). The fraction Ma1, isolated from the product Malarial-5, showed an effect in the complement assay twice times more than the one of the standard BPII, and Sl1 had similar activity to the standard. Earlier investigations have shown that polysaccharides from Malian medicinal plants activated the complement system (Diallo et al., 2003; Inngjerdingen et al., 2006 and 2012; Togola et al., 2008; Austarheim et al., 2012; Zou et al., 2014a,b). It has also been shown that the ethyl acetate extract of *Biophyton umbraculum* (syn. *B. petersianum*) showed in vitro antiplasmodial effect and also an effect in the complement...
Table 1. Yields of polysaccharides from the extractions.

<table>
<thead>
<tr>
<th>Plant material</th>
<th>Polysaccharide</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitex doniana (leaves)</td>
<td>Vd1</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Vd2</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Vd3</td>
<td>4.2</td>
</tr>
<tr>
<td>Sarcocephalus latifolius (root bark)</td>
<td>SI1</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>SI2</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>SI3</td>
<td>0.4</td>
</tr>
<tr>
<td>Argemone mexicana (aerial parts)</td>
<td>Am1</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Am2</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Am3</td>
<td>0.3</td>
</tr>
<tr>
<td>Malarial-5® (herbal tea)</td>
<td>Ma1</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Ma2</td>
<td>0.4</td>
</tr>
</tbody>
</table>


Table 2. Monosaccharide compositions (mol%) of polysaccharide fractions obtained from aqueous extracts of three antimalarial plants and the improved traditional medicine Malarial-5®.

<table>
<thead>
<tr>
<th>Monosaccharide composition</th>
<th>V. doniana (Vd)</th>
<th>S. latifolius (S)</th>
<th>A. mexicana (Am)</th>
<th>Malarial-5® (Ma)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vd1</td>
<td>Vd2</td>
<td>Vd3</td>
<td>SI1</td>
</tr>
<tr>
<td>Ara</td>
<td>14.7</td>
<td>6.6</td>
<td>1.1</td>
<td>14.4</td>
</tr>
<tr>
<td>Rha</td>
<td>5.7</td>
<td>6.5</td>
<td>4.0</td>
<td>4.5</td>
</tr>
<tr>
<td>Xyl</td>
<td>1.8</td>
<td>7.2</td>
<td>2.7</td>
<td>2.4</td>
</tr>
<tr>
<td>Man</td>
<td>4.8</td>
<td>7.4</td>
<td>Traces</td>
<td>1.5</td>
</tr>
<tr>
<td>Gal</td>
<td>13.5</td>
<td>15.1</td>
<td>10.0</td>
<td>14.8</td>
</tr>
<tr>
<td>Glc</td>
<td>19.6</td>
<td>50.4</td>
<td>69.1</td>
<td>52.9</td>
</tr>
<tr>
<td>GlcA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.9</td>
</tr>
<tr>
<td>GalA</td>
<td>39.9</td>
<td>6.8</td>
<td>13.1</td>
<td>8.6</td>
</tr>
</tbody>
</table>


Table 3. ICH50 values of the test samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>BPII</th>
<th>Vd1</th>
<th>Vd2</th>
<th>Vd3</th>
<th>SI1</th>
<th>SI2</th>
<th>SI3</th>
<th>Am1</th>
<th>Am2</th>
<th>Am3</th>
<th>Ma1</th>
<th>Ma2</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICH50 (μg/mL)</td>
<td>19.9</td>
<td>6.3</td>
<td>38.9</td>
<td>150.6</td>
<td>22.4</td>
<td>186.3</td>
<td>54.9</td>
<td>2.4</td>
<td>27.1</td>
<td>63.3</td>
<td>10.5</td>
<td>77.5</td>
</tr>
</tbody>
</table>

assay (Austarheim et al., 2016).

Glucans are often recognized by their immunostimulatory activity and complement receptor type 3 (CR3, also CD11b/CD18) is a prime candidate as β-D-glucan receptor on human monocytes, neutrophils and NK cells (also dectin-1) according to Vannucci et al. (2013). The immunostimulatory activity of various polysaccharides include glucans, pectic polysaccharides, mannan, arabinogalactans, fucoidans, galactans, hyaluronans, fructans, and xylans as reported by Ferreira et al. (2015). The complement system is a potent player in innate immunity and a major effector arm of humoral immunity. Complement activation is linked to cellular responses by the recognition of cleaved complement protein fragments by receptors on leukocytes and vascular cells. The three primary roles of complement in host defense against infection are to (1) activate an inflammatory response; (2) opsonize microbial pathogens for immune adherence; and (3) damage membranes, including lysis of susceptible organisms (Atkinson et al., 2019). Complement fixating activity has previously shown to be a good indicator for effect in the immune system by plant polysaccharides (Inngjerdingen et al., 2012). This test does not distinguish between activation and inhibition of the complement system, so we do not know if the samples have inhibited or activated the complement.
system. Medicinal plants traditionally used against inflammatory diseases and wounds containing complement inhibitors have been reported (Cazander et al., 2012).

**Measurement of nitrite oxide (NO) released from stimulated macrophages**

The ability of the polysaccharide fractions to stimulate mouse macrophages to produce NO is shown in Figure 2. NO is a good marker for macrophage activation, and its stable breakdown product nitrite can easily be detected in culture supernatants. All fractions induced a dose dependent release of NO, as measured by the quantification of its breakdown product nitrite. Among the tested samples *A. mexicana* (Am1) and *V. doniana* (Vd1) showed the highest activities by inducing the release of 2.7 and 2.8 μM of nitrite from macrophages respectively at a dose of 100 μg/mL, while the positives controls Oc50A1.I.A and LPS gave a release of 3.1 and 4.0 μM of
nitric oxide respectively. One of the important anti-parasitic chemicals generated by macrophages is nitric oxide (NO) during innate immune responses (Awasthi et al., 2003).

Plant-derived polysaccharides are potent immunomodulatory substances, and have been shown to be clinically therapeutics, eg, lentil. Previous authors reported that a variety of beneficial pharmacological effects of plant polysaccharides were attributed to their ability to modulate macrophage immune function (Yu et al., 2017). Some earlier studies supported the proposition that the production of nitric oxide by macrophages plays a crucial role in the control of parasitaemia at the initial periods of blood stage malarial infection (Awasthi et al., 2003). However, the ethyl acetate extract of *B. umbraculum* which revealed *in vitro* antiplasmodial effect, but gave an inhibition of macrophage activation (Austarheim et al., 2016).

### Conclusion

All polysaccharide fractions from *A. mexicana*, *S. latofoliis*, *V. doniana* and Malarial-5™ contain pectic type polymers as well as glucans. These polysaccharides displayed immunomodulatory properties primarily as determined by the complement assay. The characterization of the polysaccharides acting on the immune system explains more the effectiveness of aqueous extracts and gives an additional justification for the traditional form. These results could be used as a new approach in the management of malaria. Therefore further investigations will be undertaken on *A. mexicana* that showed the highest immunomodulatory activity.

### CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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