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## FROM ETHNOPHARMACOLOGY TO GREEN CHEMISTRY: “DAWILOU” A TRADITIONAL HERBAL MIXTURE

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### ABSTRACT

Since long times, human employs medicinal plants to treat illnesses, using frequently oily or aqueous preparations. Such uses are one of the most important principles of green chemistry which called 'green solvents'. Within the context of clean and green pharmaceutical industry, ethnopharmacological preparations must to be investigated to reduce toxicity of chemicals and solvents. 'Dawilou' is an ancient preparation used in Constantine as an Algerian Traditional Medicine (ATM); it is an aqueous herbal mixture of four spices: lesser galangal rhizome, ginger rhizome, cinnamon bark and clove buds. It is used to prevent or treat cold disease and to give some energy to workers in ancient times. The decoction was studied for its qualitative chemical composition using phytochemical screening; it was found that the extract is rich in chemical constituents: phenolics, terpenoids and alkaloids. Biological activity of 'Dawilou' against clinical isolated bacteria was investigated also and found to have moderate activity *in vitro* because of bacterial nature. So, the *in vivo* study is necessary to complete the study and evaluate immunostimulating activity of the extract which is empirically proved.

**Keywords:** 'Dawilou', Decoction, Spices, Phytochemical screening, Antibacterial effect, Immunostimulating.

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### INTRODUCTION

Traditional medicines in all civilizations are very rich but not well scientifically investigated. However, the increasing need of new active molecules extracted by environmentally friendly simple processes encourage the combination between 'natural ethnopharmacological resources' and 'green chemistry'.<sup>1-3</sup> The use of water as an alternative of toxic solvents in green chemistry principles<sup>4</sup> is an ancient ethnopharmacological practice to prepare

infusions and decoctions.<sup>5</sup> The renewal use of water is due to its particularities, it is non toxic, non flammable, ubiquitous, it is the basis of all chemical reactions in biological systems and it can extract either hydrophilic and hydrophobic molecules according to conditions.<sup>4</sup>

Algerian Traditional Medicine (ATM) contains natural remedies, most of them are endemic plants (Mediterranean or Saharan), culinary herbs, and other ingredients given from Asian and African areas such as spices.<sup>5</sup> ATM is used

according to empirical knowledge of populations and under the guidance of Islamic Medicine found in Quran and Sunna, which support the use of some aromatic plants.

In Constantine, spices are used separately or in combination in some meals, cakes or as a medicine. "Dawilou" is one of many traditional formulations found in ATM; it is an aqueous mixture of four spices: ginger (*Zingiber officinale*), galangal (*Alpinia officinarum*), Ceylon cinnamon (*Cinnamomum zeylanicum*) and clove buds (*Syzygium aromaticum*), used in periods of cold to prevent infections and give energy to workers. These spices are widely studied for many biological activities.<sup>6-11</sup>

The aim of this study is a scientifically chemical investigation and antibacterial evaluation of traditional formulation -'Dawilou'- an aqueous extract able to kill bacteria, within the context of green chemistry, when using water only as an alternative of toxic solvents to provide extract with several biological activities.

## MATERIALS AND METHODS

### Extracts Preparation

Ceylon cinnamon bark, clove buds, ginger and galangal were purchased from local market. From each plant 2.5g (10g of the mixture) were extracted with 250ml of distilled water as therapeutic dose.<sup>5</sup> Decoction was prepared by introducing plant and water in glass beaker till ebullition at 100°C for 30 min. The final concentration in cultural media was 4mg/ml which is the highest concentration.

### Phytochemical Screening

Aqueous plants extract was screened for the presence of secondary metabolites: saponins, flavonoids, triterpenes/steroids, proanthocyanidols, tannins, phenolic acids, anthocyanins, coumarins and alkaloids, and one test for primary metabolite (starch), in test tubes using a variety of reagents for detection as colorimetric methods described by many works.<sup>6, 12, 13</sup>

### Antibacterial Activity

Clinical Gram Positive and Gram Negative bacteria were selected for evaluation of aqueous extract antibacterial activity. Isolates were collected from different materials: pus, blood, urine, gastric, vaginal and sperm samples, wound, spine liquid, tracheal and vesicle sound. They were obtained from the Laboratory of Bacteriology-Hospital of Constantine (C.H.U. de Constantine), and others were obtained from the Laboratory of Bacteriology of Institut Pasteur d'Alger-Annexe of M'sila, Algeria.

Antibacterial activity was evaluated by the agar dilution method<sup>14</sup>, using Mueller-Hinton Agar (MHA). The Minimum Inhibitory Concentration (MIC) was the lowest concentration able to inhibit bacterial growth. Dilutions prepared from aqueous extracts were 2 and 1mg/ml. A blank containing only MHA served as a control. Petri dishes were inoculated with bacterial strains (0.5 Mc Farland) and were incubated at 37°C during 24 h. Each test was repeated three times.

## RESULTS AND DISCUSSION

Obtained decoction of plants mixture was subjected to phytochemical screening which revealed the presence of a variety of molecules according to colorimetric methods: saponins (presence of foam), flavonoids (pink colour), condensed tannins (brick red), catechic tannins (black green), phenolic acids (yellow), coumarins (one yellow spot on the paper under UV light), triterpenoids (pink orange), unsaturated steroids (brick red) and alkaloids (dark precipitate) as secondary metabolites, and the presence of starch (dark blue) as primary metabolite (Table1).

As presented in Table 2, the extract was able to inhibit some strains, weakening others and has no effect against the rest of bacteria. The first group (A) of bacteria was more sensitive to extract than the second one (B); it may be due to the nature of strains or the chemical composition of decoction. Bacteria of group A were given from city of M'sila, where patients don't use a lot of antibiotics in general; so strains are wild and less resistant. However, bacteria of group B

were given from city of Constantine, where patients use many antibiotics; subsequently strains are more resistant and most of them are nosocomial such as *Xanthomonas maltophilia*, or extended-spectrum  $\beta$ -lactamase-producing as *Serratia* sp.

Bacterial resistance to the extract may due to chemical composition of the decoction; it contains some primary metabolites such as starch which is biodegradable and may act as bacterial growth stimulating<sup>13</sup>, which has reverse the effect of the rest of components known to have antimicrobial effect.<sup>6, 9, 15</sup> This extract when intake as a tea in cold periods may have direct effect against germs attacks, or their metabolites, after ingestion, may strengthen immune system (immunostimulant)<sup>16-18</sup>, since it contains spices that warm the body and expel cold.<sup>19</sup> Chemical compounds in decoction mixture are various and different but act synergistically<sup>20</sup>; therefore the extract could operate on different targets in bacterial cells and human body.

## CONCLUSION

In conclusion, the herbal formulation called 'Dawilou' decoction which composed by ginger, galangal, cinnamon and clove, and used successfully in Constantine (Algeria) against cold diseases, was found to have rich chemical composition of phenolics, terpenoids and

alkaloids, which act together to kill bacteria or enhance immune system. When testing *in vitro* against gram positive and gram negative bacteria, the decoction has moderate effect due to low therapeutic dose, non toxic and acts slowly and efficiently. The antibacterial activity of the extract depends not only on bacterial strains, but also on the illness and the patient status if same strains have different responses.

Such *in vitro* study need to be continued by an *in vivo* study to improve these results and to find the mode of action as an immunostimulating agent. Aqueous extracts were used in therapeutics in the past as natural phytochemical association, but remain efficient till nowadays within new context called 'green chemistry', for the fruitful future pharmaceutical bio-industry, using clean and green methods.

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**Table 1:** Phytochemical screening and Total phenolic content of 'Dawilou' decoction

Chemical substances	Tests or Reagent	Aqueous extract
Saponins	Shaking and forming foam	+
Flavonoids	Concentred HCl + Mg <sup>2+</sup>	±
Pronthocyanidols	Concentred HCl	+
Tannins	FeCl <sub>3</sub> (1% in MeOH) + NaCl (10%)	+
Phenolic acids	Colouring Indicator (BTB)	+
Anthocyanins	HCl (2N)	-
Coumarins	Paper humid by NaOH/UV light	+
Triterpenes / Steroids	Liebermann- Burschard	+/+
Alkaloids	Dragendorff	+
Starch	Iodine	+

(+): presence, (±): small quantity, (-): absence

Table 2: Minimum Inhibitory Concentration of 'Dawilou' decoction

Bacterial strains		Minimum Inhibitory Concentration (mg/ml)
A	<i>Pseudomonas aeruginosa</i>	+
	<i>Klebsiella sp</i>	-
	<i>Escherichia coli</i>	±
	<i>Staphylococcus aureus</i>	±
B	<i>Xanthomonas maltophilia</i>	+
	<i>Morganella morganii</i>	+
	<i>Serratia sp</i>	+
	<i>Staphylococcus albus</i>	+

MIC $\geq$ 4mg/ml, (+): presence, (-): absence and (±): weak bacterial growth.

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