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Phytochemical Study and In Vitro Biological Assays on *Zingiber officinal*: A Widely Used Spice †

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† Presented at the 1st International Electronic Conference on Biological Diversity, Ecology and Evolution, 15–31 March 2021; Available online: https://bdee2021.sciforum.net/.

Abstract: *Zingiber officinal* is a widely used plant in cooking as well as traditional remedy in prevention of digestive conditions and disorders, cancer, inflammatory disease, antiseptic. In recent years several studies are conducted on botanical, chemical and toxicological parts of this plant in order to prove a concordance between traditional and medicinal knowledge. In fact, plant based metabolites is an interdisciplinary field, as it requires knowledge of botanic, pharmacology, food, chemical, clinical, preclinical, herbal drug technology, microbiology etc. There is an urgent need to explore and investigate the innovations, current shortcomings, future challenges explore and convey the key concepts for understanding the assessment of plant based metabolites in therapeutically caring. Furthermore, Drug discovery from plants goes through different strategies: empirical approach like ethno botanical and pharmalogical studies, and chimiotaxonomical one like choosing certain secondary metabolites family phenols, flavones, terpens... In this case, the present work is a contribution in the evaluation of *Zingiber officinal's* rhizome percolate potential in polyphenols, flavonoïdes, in vitro antioxidant test Ferric reducing *antioxidant* power FRAP, antibacterial activity against several gram + (*S. aureus)* and gram-(*E. coli*, *P. aeruginonose, K. pneumoniae)* referential strains and antifungal activity (*Candida albicans, Aspergillus niger,)* were tested using disk diffusion method, which reveled a very interesting dose-depending activity (from 16 to 26 mm) against *S. aureus*, *P. aeruginonose and K. pneumoniae,* similar to used standard Gentamicin GN, whereas fungi exhibit less sensitivity with 10 mm of inhibition and *E. coli* was resistant to crud ginger extract.

Keywords: *Zingeber officinal*; phenols; antioxidant activity; antimicrobial activity

Citation: Mouas, T.N.; Kabouche, Z.; Boucharka, A.; Messaoud, A. Phytochemical Study and In Vitro Biological Assays on *Zingiber officinal*: A Widely Used Spice. *Proceedings* **2021**, *68*, x. https://doi.org/10.3390/xxxxx

1. Introduction

Ginger is the common name for the whole or cut rhizome (underground stems) of the plant Zingiber officinal Roscoe, Fam. Zingiberaceae, it is cultivated essentially in tropical regions. Ginger has been widely used in ethnomedicine to treat nausea and vomiting in pregnant women [1], its main active compounds are phenols namely gingerols [2–5]. However, many other therapeutic effects have been reported: analgesic, antiinflammatory, and metabolic (antidiabetic and hypolipidemic) actions. Ref. [6] gastrointestinal diseases, cancer, and hypertension [7–10], antiemetic properties of ginger [11,12]. In addition, toxicity studies in rodents, with high doses of different ginger preparations (i.e., ginger extracts and ginger powder), showed no signs of toxicity [13], even at daily doses of up to 1000 mg/kg body weight [14], since Ginger has a long history of use as a food and medicine and is "generally recognized as safe" (GRAS) as a food flavoring by the U.S. Food and Drug Administration. Which make it among the top-selling herbal dietary supplements in 2017 [15]. Consequently, it could be very interesting to include this nutraceutical in humans' daily food.

2. Experiments

2.1. Plant Materials

Zingiber officinal dried rhizomes was bought and identified in a herbal shop.

All used chemicals are of analytical quality, and strains ATCC referenced:

- *Staphylococcus aureus* ATCC 25923
- *Escherichia coli* ATCC 25922
- *Pseudomonas aeruginonose ATCC 27853*
- *Klebsiella pneumoniae ATCC700603*.

2.2. Extraction

The plant rihzoms are grounded into powder using a mortar and a pestle, then extracted for 2 h with Sohxlet apparatus and ethanol as extraction solvent.

The percolate is filtered using a Whatman paper N°4, and evaporated at 40 °C under reduced pressure, maintained with a vacuum pump, to give the crud ethanol extract conserved aseptically in the freezer for future uses in the quantitative analysis.

Yields are calculated according to the following formula:

$$\text{Yield } \% = (\text{Crude extract mass/powder mass}) \times 100$$

2.3. Total Polyphenols Content

0.2 mL of sample was firstly mixed with 1 ml of diluted Folin–Ciocalteu reagent (5/10 H_2O) by vortexing. After that, 0.75 mL of Na_2CO_3 (7.5%) are added. Then, the reaction mixtures are further incubated for 2 h at room temperature in the dark, and finally, the absorbed optical density is recorded at the wavelength of 765 nm [16,17].

2.4. Total Flavonoid Content

0.4 mL of diluted sample with 1 ml ethanol is separately mixed with 1 mL of 2% aluminum chloride methanol solution. After incubation at room temperature for 15 min, the absorbance of the reaction mixture is measured at 430 nm with spectrophotometer [18].

2.5. The Antioxidant Activity Analysis

The antioxidant activity was measured using reducing power test following Oyaizu [19] protocol with slight modifications. To 10 µL of extracts, 40 µL of phosphate buffer pH (6.6) and 50 µL of potassium ferricyanure $K_3Fe(CN)_6$ 1% (1 g $K_3Fe(CN)_6$ in H_2O) are added. After 20 min of incubation at 50 °C, 50µL of trichloracetic acid (TCA) at 10%, 40 µL of water and 10 µL ferric chloride $FeCl_3$ (0.1% in H_2O) are added. The solution absorbance is measured at 700 nm. For the blank replace the extracts with methanol. Ascorbic acid, is used as standard.

2.6. The Antimicrobial Activity Analysis

The antimicrobial susceptibility and resistance tests of our extracts were carried out according to the Agar disk-diffusion testing developed in 1940 [20].

Discs (Whatman No. 1, 6 mm diameter) are impregnated with each extract and then applied to the surface of the agar plates which have been seeded by spreading the microbial suspension. The seeding is carried out in such a way to ensure a homogeneous distribution of the bacteria. The petri dishes are incubated during 24 h at the appropriate temperature 37 °C in the laboratory oven, and the resulting inhibition zone diameter was measured in millimeters using a ruler.

Antimicrobial activity is determined in terms of the diameter of the inhibition zone produced around the discs.

2.7. Statistical Analysis

Sampling and analyses were performed in triplicate, and the data are presented as mean ± standard deviation (S.D.). Statistical analysis was performed using Microsoft Office Excel 2008 ($p < 0.05$).

3. Results and Discussion

3.1. Total Phenol and Flavonoid Compound Content Results

The total phenol content showed total polyphenols content of (11.5 ± 0.12) μg EGA/mg DE, and total flavonoids content of (2.12 ± 0.17) μg QE/mg for rhizomes'' ethanol percolate.

3.2. Evaluation of Biological Activities

3.2.1. Antioxidant Activity

The FRAP assay exhibit a very high reducing potency for tested extract with an ABS = 3 for 100 μg/mL of extract, similar to referential used standard ascorbic acid at the same concentration, same results were previously reported [21,22]

3.2.2. Antimicrobial Activity Results

- **Antibacterial test**

The diameters results of the growth inhibition zones exhibit an important dose-depending antibacterial potential, thus we noticed:

- 16 mm to 27 mm of inhibition zones for *P. aeruginonose* treated with 13.25 to 1000 μg/mL respectively of extract,
- 16 mm to 26 mm of inhibition zone for *Staphylococcus aureus* treated with 125 to 1000 μg/mL of extract,
- 16 mm to 26 mm of inhibition zone for *K. pneumoniae* treated with for 62.5 to 1000 μg/mL of extract.
- *E.coli* showed up as resistant strain.

Gentamicin (10 μg/ disc) and was used as positive control. Obtained results are in nice agreement with literature [23–25].

- **Antifungal activity test**

Antifungal activity against *C. albican* and *A. niger* tested fungus reveal a moderate inhibition about 10 mm and 11 mm respectively for 1000 μg/mL of extract.

4. Conclusions

In the present work a widely used spice in culinary field as well as traditional remedy *Zingeber officinal* rhizomes'' methanol percolate was investigated through its in vitro antioxidant and antimicrobial activities assessment. Qualitative and quantitative analysis methods were used: Sohxlet extraction, thin layer chromatography (TLC) and UV spectroscopy to evaluate its phenols potent. The antioxidant effect of studied plant was evaluated by the FRAP test, the antimicrobial activity was evaluated by disk diffusion method for three Gram (-) bacteria (*E. coli*, *P. aeruginonose, K. pneumoniae*), one bacteria Gram (+) (*Staphylococcus aureus*) and two funguses: *Candida albicans, Aspergillus niger*. and exhibit an interesting antibacterial effect for *P. aeruginonose, K. pneumoniaeand Staphylococcus aureus,* similar to used standard Gentamicin GN. Therefore, it may be considered, as an efficient nutraceutical and functional food for treating gastrointestinal disorder.

Author Contributions: M.T.N. conceived and designed the experiments, analyzed the data and wrote the paper; B.A. and M.A. performed the experiments; Z.K. scientific assistance. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Aknowledgments: Authors would like to thank Algerian Ministry of Higher Education and Scientific Research DGEFS, and the Algerian Directorate General for Scientific Research and Technological Development DGRSDT for financial fund.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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