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In vitro bioguided trials on *Cinnamomum zeylanicum* percolate as target antimicrobial agent

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Abstract: C*innamomum zeylanicum* gender, a wildly used plant as spice and medicinal plant, is investigated in both chemical and biological fields, since development of bio-guided trials on medicinal plants therapeutic target has increased recent years through pharmacology which is interested in discovering new molecules expressing a therapeutic activity and development of useful drugs by selecting the most active fraction and isolating the active compound responsible of the therapeutically effect.

Therefore, identification and quantification analysis of main bioactive compounds were performed in order to undergo bio-guided tests using several solvents' polarities to evaluate its *in vitro* antimicrobial potential. To achieve this objective, qualitative and quantitative methods were used to identify bioactive compounds of the obtained extracts. The *in vitro screening of* antimicrobial effect was evaluated on 10 bacteria and 2 funguses by disk diffusion method which gave almost very interesting results for all tested pathogens in addition to richness in secondary metabolites.

Keywords: Medicinal plants; bioactive compounds; bioguide assays; antibacterial activity

1. Introduction

Cinnamon (*Cinnamomum spp., Lauraceae* family) includes more than 250 evergreen trees spread mainly in Asia, China, and Australia [1]. The most studied types of cinnamon are Cinnamomum verum and Cinnamomum zeylanicum [2,3,4], and the main components of cinnamon bark is (E)-Cinnamaldehyde (49.9%) [5], one of possible side effects of cinnamaldehyde is hypersensitivity [6]. However, reports of allergic contact dermatitis and stomatitis due to cinnamon are rare [7, 8, 9,10,11]. According to statistics, 30% of the populations in industrialized countries develop food borne infections each year [12]. Therefore, there have been growing concerns regarding food safety, attracting attention to the use of natural antimicrobial agents for the proper control of food borne pathogenic and spoilage bacteria [13,14]. Recently, food industries have become more interested in the use of natural preservatives [15].

Natural additives such as essential oils (EOs) and herbal extracts have been considered economically for their medicinal effects, low toxicity, and cost-effectiveness [16,17]

Microorganisms are the main cause of the high prevalence of food borne diseases worldwide [18]. Most cases of food poisoning are associated with bacterial contamination, especially gram-negative bacteria such as *Salmonella typhi, Escherichia coli,* and *Pseudomonas aeruginosa* and gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus cereus* [19]

Cinnamon is widely used in the food, cosmetic, and pharmaceutical industries. Considering its fragrance and ability to eliminate mouth odor, cinnamon is also added to chewing gum as a spice [20,21]. Previous studies have shown that cinnamon and its components (cinnamaldehyde and eugenol) which possess antibacterial activity against *Parahemolyticus, Staphylococcus epidermis, Enterococus faecalis, Pseudomonas aeruginosa, Salmonella sp., Staphylococcus aureus* and *Escherichia coli* [22]. Moreover, cinnamon oil has strong hypocholesterolaemic, antioxidant, analgesic, antiulcer and anticandidial activities [23,24]. Furthermore, the antimicrobial properties of cinnamon make this spice beneficial in the production of films and edible coatings, which are used for the packaging of various food products [25, 26].

The present study aimed to investigate the *in vitro* antimicrobial effects of cinnamon bark extract using bio-guided fractioning and assays.

2. Experiments

2.1. Plant materials

Cinnamon bark was identified and bought in a local herbal shop.

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

Gram (-) bacteria:*Escherichia coli* ATTC 25922, *Pseudomonas aeruginosa*: ATTC 27853, *Pseudomonas aeruginosa*: ATTC 2330, *Pseudomonas aeruginosa*: ATTC1616, *Klebsiella pneumoniae*: ATTC 700603, *Acinetobacter baumannii*: 1625, *Salmonella*: clinical strain, *Citrobacter freundii*: 22, *Enterobacter aerogenes*: clinical strain, *Proteus Sp*: clinical strain, Gram (+)bacteria: *Staphylococcus aureus 2S*: ATTC 43300, Mushrooms: *Candida albicans*: clinical strain, *Rhizopus oryzae*: M491890.1.

2.2 Solid- liquid extraction

The plant dried bark is grounded into powder using a mortar and a pestle, then extracted for 2h with Soxhlet 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:

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

2.3 Liquid- liquid extraction

Cinnamon crud ethanol extract is subjected to bio-guided extraction using separating funnel and alternately different solvent polarity as: water, chloroform, ethyl acetate and n-butanol. Four extracts are obtained, filtered on sodium sulfate, then dried and stoked in the freezer.

2.4. Bioactive compounds content:

TLC screening is performed to choose most interesting and rich extract profile to analyze, bioactive compounds by means of total phenols and flavonoids were quantified for obtained extracts: chloroform, ethyl acetate, n-butanol, aqueous extract.

- **Total polyphenols content**

0.2 ml of sample was firstly mixed with 1ml of diluted Folin–Ciocalteu reagent (5/10 H_2O) by vortexing. After that, 0,75ml of Na_2CO_3 (7,5%) are added. Then, the reaction mixtures are further incubated for 2 hours at room temperature in the dark, and finally, the absorbed optical density is recorded at the wavelength of 765 nm [27,28].

- **Total flavonoid content**

0,4 ml of diluted sample with 1ml 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 [29].

2.5 The antimicrobial activity

The antimicrobial susceptibility and resistance tests of our extracts were carried out according to the Agar disc-diffusion testing developed in 1940 [30]

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 hours at the appropriate temperature 37C° 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

TLC screening reveled chloroform extracts as the richest in secondary metabolites followed by acetate extract then n-butanol extract, aqueous extract is the poorest one, consequently it was excluded from quantitave analysis and antimicrobial activity.

3.1. T**otal phenol and flavonoid compound content results**

- The total phenol content showed total polyphenols content of (300±0.01) µg EGA/mg DE, and total flavonoids content of (158±0.1) µg QE/mg for ethyl acetate extract.
- The total phenol content showed total polyphenols content of (28±0.15) µg EGA/mg DE, and total flavonoids content of (2.5±0.2) µg QE/mg for n-butanol extract.
- Total flavonoids content of (50±0.05) µg QE/mg for chloroform extract.

3.2 Antimicrobial activity results

3.2.1Antibacterial test

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

For chloroform extract,

- 10mm to 13mm of inhibition zones for *Staphylococcus aureus* 2S treated with 15.625 to 500 µg/ml of extract.
- 10mm of inhibition zone for *Enterobacter* treated with 15.625µg/ml of extract,
- 10mm to 18mm of inhibition zone for *Citrobacter Freudii* treated with 15.625 to 62.5 µg/ml of extract.
- 15mm to 30mm of inhibition zone for *E.Coli* treated with 31.25 to 250 µg/ml of extract.
-10mm to 28mm of inhibition zone for *Acinetobacter* treated with 62.5 to 500 µg/ml of extract.
-10mm to 18mm of inhibition zone for *Kleb pneumoniae* treated with 15.625 to 1000 µg/ml of extract.
-10mm to 15mm of inhibition zone for *Pseudomonas aeruginosa 1* treated with 15.625 to 500 µg/ml of extract.

-10mm to 24mm of inhibition zone for *Pseudomonas aeruginosa 2* treated with 15.625 to 1000 µg/ml of extract.

Salmonella, Proteus SP and *Pseudomonas aeruginosa 3*, showed up as resistant strains to chloroform extract.

For ethyl acetate extract,

- 8mm to 15mm of inhibition zone for *Enterobacter* treated with 31.25 to 500µg/ml of extract,
- 10mm to 22mm of inhibition zone for *Citrobacter Freudii* treated with 15.625 to 1000µg/ml of extract.
- *8*mm to 11mm of inhibition zone for *Proteus* treated with 31.25 to 250 µg/ml of extract.

-19mm to 20mm of inhibition zone for *Kleb pneumoniae* treated with 31.25 to 500 µg/ml of extract.

-8mm to 20mm of inhibition zone for *Pseudomonas aeruginosa 1* treated with 15.625 to 1000 µg/ml of extract.

-10mm to 15mm of inhibition zone for *Pseudomonas aeruginosa 2* treated with 15.625 to 500 µg/ml of extract.

Salmonella, Acinetobacter baumannii, E.coli, Pseudomonas aeruginosa and *Staphylococcus aureus 2*, showed up as resistant strains to chloroform extract.

For acetate n-butanol extract,

- 8mm to 18mm of inhibition zone for *Enterobacter* treated with 15.625 to 500µg/ml of extract,
- 11mm to 13mm of inhibition zone for *Citrobacter Freudii* treated with 31.25 to 500µg/ml of extract.
- *8*mm to 19mm of inhibition zone for *Proteus* treated with 31.25 to 500 µg/ml of extract.

-8mm to 12mm of inhibition zone for *Kleb pneumoniae* treated with 15.625 to 125 µg/ml of extract.

-10mm to 22mm of inhibition zone for *Pseudomonas aeruginosa 1* treated with 15.625 to 62.5 µg/ml of extract.

-8mm of inhibition zone for *Pseudomonas aeruginosa 2* treated with 15.625 µg/ml of extract.

Salmonella, Acinetobacter baumannii, E.coli, Pseudomonas aeruginosa 3, Staphylococcus aureus 2S, showed up as resistant strains to chloroform extract.

Gentamicine GN(10µg /disc), Nalidixique NA (30µg/disc) and Imipeneme IP (10µg/disc) were used as positive control.

Obtained results are in nice agreement with literature [31-34].

3.2.2 Antifungal activity test

Antifungal activity against *C.albican* fungi revealed varying inhibition capacity about:

- 14-23mm for 62.5-250 µg/ml of chloroform extract.
- 11-18mm for 31.25-1000 µg/ml of acetate ethyl extract.
- 10-12mm for 62.5-1000 µg/ml of n-butanol extract.

Rhizopus oryzae was resistant to all tested extracts.

Obtained results are in nice agreement with literature [35-36]

4. Conclusions

In the present work, *Cinnamomum zeylanicum* a wildly cultivated and used spice, famous in all pharmacopeias for its therapeutic effect was phytochemically and biologically assessed, by subjecting its ethanol percolate to a bio-guided fractioning using different solvent polarities, identification and quantification of secondary metabolites by layer chromatography (TLC) and UV spectroscopy, undergoing *in vitro* biological trials by mean of anti bacterial and antifungal activities on several referential strains, which gave an important inhibiting activity against Gram (+) bacteria :

Staphylococcus aureus, seven Gram (-) bacteria : *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Proteus Sp*, and one fungi : *Candida albicans*. Obtained results, open large perspectives on bioguided fractioning in order to identify bioactive molecules responsible of therapeutic effect and pharmaceutical enhancement of studied spice promoting it as an efficient nutraceutical for treating human microbial resistant phenomenon.

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