BioActivity Screening Services

Matula Tea

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1. Methodology

1.1 General Information

The prepared product was provided by the client. This was a herbal tea, known as "Matula" tea. The product was filter sterilized and used as is in all the assays. All assays were performed in duplicate. Sterile methods and equipment was used throughout all the assays where needed.

1.2 First Phase Anti-bacterial Screening

The product was tested for activity against three different bacteria. These bacteria were *Staphylococcus aureus* (ATCC 25923), *Helicobacter pylorii*, and two strains of *Escherichia coli* (ATCC 35218 and ATCC 25922). *S. aureus* and *H. pylori* were grown up over night in a slime broth, while the two *E. coli* strains were grown up overnight in nutrient broth.

The overnight cultures of bacteria were then incubated overnight in the presence of three different concentrations of Matula tea, namely 50%, 33%, and 20%. A positive control culture of each bacteria and each concentration was also incubated overnight in the presence of matching concentrations of sterile water. This was done to compensate for the water component present in the tea. Negative controls (media with the respected concentrations of sterile water) were also included in this experiment.

A chemi-luminescent assay was used to determine the amount of viable bacteria cells in each instance. Background luminescence was eliminated by deducting the negative controls from their corresponding results. The difference between each positive control and its equivalent of bacteria cells in the presence of Matula tea was expressed as a percentage of inhibition.

1.4 Second Phase Cell Viability Testing

The product was tested for its effect on the viability of PBMCs at three different concentrations. The effect of Matula tea was compared to the effect of filter sterilized rooibos tea, since rooibos tea is generally regarded as safe. Three different concentrations of the product and rooibos tea was used, namely 5%, 2% and 1%.

Peripheral blood mononuclear cells were isolated via density centrifugation from fresh whole blood from a healthy donor. The isolated PBMCs were then suspended in complete medium and diluted with complete medium to an approximate final concentration of 1 million cells per milliliter. The PBMCs were incubated overnight in the presence of the three different concentrations of Matula tea and rooibos tea.

A chemi-luminescent assay was used to determine the amount of viable cells in each instance. Background luminescence was eliminated by deducting the negative controls from their corresponding results. The difference between each positive control and its equivalent of cells in the presence of Matula and rooibos tea was expressed as a percentage cell death.

1.5 Second Phase Anti-bacterial screening

After the first phase of anti-bacterial evaluation it became apparent that the product displayed significant anti-bacterial activity. To further explore the commercial value of this activity the product was tested for activity against the problematic methicillin resistant *Staphylococcus aureus* (MRSA). Furthermore and based on testimonials, the product was also tested for antifungal activity, in specific against *Candida albicans*.

MRSA and *C. albicans were* grown up overnight in nutrient broth. The overnight culture of MRSA was then incubated once again overnight in the presence of four different concentrations of Matula tea, namely 50%, 20%, 10% and 5%. In the case of the *C. albicans* culture, Matula tea was only used at 50%, 25%, and 5% concentrations. A positive control culture of each culture and each concentration was also incubated overnight in the presence of matching concentrations of sterile water. This was done to compensate for the water component present in the tea. Negative controls (media with the respected concentrations of sterile water) were also included in this experiment.

A chemi-luminescent assay was used to determine the amount of viable microbic cells in each instance. Background luminescence was eliminated by deducting the negative controls from their corresponding results. The difference between each positive control and its equivalent of microbial cells in the presence of Matula tea was expressed as a percentage of inhibition.

2. Results

2.1 First Phase Anti-bacterial Screening

2.1.1. Organism: *Staphylococcus aureus* ATCC 25923 (Gram +)

Concentration of Product	Percentage Inhibition
50%	94
33%	35
20%	44

2.1.2. Organism: *Escherichia coli* ATCC 35218 (Gram -)

Concentration of Product	Percentage Inhibition
50%	80
33%	69
20%	44

2.1.3. Organism: *Escherichia coli* ATCC 25922 (Gram -)

Concentration of Product	Percentage Inhibition
50%	86
33%	74
20%	56

2.1.4. Organism: *Helicobacter pylorii* Clinical Isolate (Gram -)

Concentration of Product	Percentage Inhibition
50%	93
33%	92
20%	74

2.3 Second Phase Cell Viability Testing

2.3.1. PBMCs death in presence of Matula Tea

Concentration of Matula Tea	Percentage Cell Death
5%	10
2%	28
1%	22

2.3.2. PBMCs death in presence of Rooibos Tea

Concentration of Rooibos Tea	Percentage Cell Death
5%	10
2%	32
1%	37

2.4 Second Phase Anti-bacterial screening

2.4.2. Organism: *Candida albicans*

Concentration of Matula tea	Percentage Inhibition
50%	93
25%	84
5%	70

3. Discussion

3.1 First Phase Anti-bacterial Screening

The product displays a significant anti-bacterial effect against both Gram + and Gram – bacteria. Furthermore, it displays potent activity against Helicobacter pylori, the major cause of stomach ulcers.

3.3 Second Phase Cell Viability Testing

The effects of Matula tea on the viability of PBMCs is similar, and even slightly less pronounced, when compared to the effect of Rooibos tea on the same cell type. Since Rooibos tea is generally regarded as a safe herbal tea, it was used as a comparison in this experiment.

3.4 Second Phase Anti-bacterial screening

. Furthermore, Matula tea had a significant inhibitory effect on *C. albicans* across the concentration range used in the experiment.

Signed:

Prof. P.J.D. Bouic
Chief Technical Officer

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W.J. Kriek
BioActivity Screening Manager

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