

Brine-Shrimp Lethality Bioassay of different extracts of the medicinal plant matricaria (chamomilla) flowers

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Research Article

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Abstract

The present study aimed to throw some light on phytochemical constituents of *Matricaria* (chamomile) flowers. The grinded flowers were extracted successively by petroleum ether (40-60°C), chloroform, 95% ethanol, and ethyl acetate; using soxhlet apparatus respectively. The extracts were concentrated under vacuum (rotatory evaporator). Phytochemical screening of *matricaria* flowers, were performed using standard methods, showed the presence of alkaloids, flavonoids, terpenoids, saponins, and anthraquinone. The 95% ethanolic crude extract was subjected to column chromatography and nine fractions were collected. Fractions 1-6 have the same R_f value, (using thin layer pre-coated silica gel (0.25cm). were combined and subjected to spectroscopic analysis; infrared spectra showed absorption at 1860, 1637 and 805 cm⁻¹ indicating the presence of C=O, C=C, and C-O groups respectively. Ultra violet absorption at 250-283nm assign for the presence of flavonoids, (lit. 250-280). Finally GC/MS spectra performed the presence flavonoids. Crude extracts (ethanol, methanol, petroleum ether, and chloroform) were investigated for antimicrobial activities against four strains of bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and two fungi: *Aspergillus niger*, and *Candida albicans*) with disc diffusion method, the diameter of growth inhibition zone ranged 15-22mm. The distilled essential oil (hydro distillation) of *matricaria* flowers was subjected to GC/MS, a Number of aliphatic compounds were detected e.g. 2, 3 heptadione and 3-hexa none, 2, 5 dimethyl.

Introduction

Natural products from plants are important sources of drugs as several important medicines have been isolated and produced to cure ailments [1-5].

The pharmacological evaluation of extracts of organisms and pure isolates is an area of an essential aspect of the drug discovery process and developments in the techniques have substantially transformed this fact of natural product chemistry [1&3]. "Secondary testing" procedures need more detailed testing in order to select compounds for clinical trials [6].

The main needs of primary bioassay screen should meet the following:

1. The bioassay results should predict some type of therapeutic potential
2. Potentially useful pharmacological activity should not go undetected even though the activity may be either unexpected or unique.
3. The expected nature of the activity should be indicated for further research
4. The primary bioassay screening test should be adapted for impurities present in a crude extract.
5. The bioassay procedure should be unbiased and it should allow for the coding of all samples, including both "known reference materials (standards) and unknown test samples and the results obtained should be repeatable.
6. The use of crude materials and pure isolates should be carefully studied.
7. The single bioassay screen should as minimum as possible (1-2g) of the crude dry natural material (plant or animal extract).
8. Avoid expensive equipment or a sophisticated laboratory environment The procedure should be compatible with the use of dimethyl sulfoxide (DM50).
9. The procedure should be simple enough to be taught easily to laboratory technicians [6] so that highly trained and qualified researchers are not required for the routine operation of the bioassay program [6&7]

The choice of the screening approach to be adopted generally depends on the target disease as well as on the available information about the target organism (plant, marine animal, etc.) to be studied. For example if a plant has a ethanopharmacological history of use against a particular disease, then one would logically use a specific bioassay technique (single goal screening) which can predict the reputed therapeutic activity in order lead which is responsible for that biological activity[7&8].

Chamomile (*Matricaria chamomile* L.) is a well-known medicinal plant species from the Asteraceae family often referred to as the "star among medicinal species."

German chamomile is used in herbal medicine for a sore stomach, irritable bowel syndrome, and as a gentle sleep aid. It is also used as a mild laxative and is anti-inflammatory and bactericidal. It is Acaricides used against certain mites, such as *Psoroptes cuniculi*. [9].

Botanical classification of *Matricaria recutita* or German chamomile:

| | |
|---------------------------|----------------------------|
| Scientific classification | |
| Kingdom: | Plantae |
| (unranked): | Angiosperms |
| (unranked): | Eudicots |
| (unranked): | Asterids |
| Order: | Asterales |
| Family: | Asteraceae |
| Tribe: | Anthemideae |
| Genus: | <i>Matricaria</i> L., 1753 |

Food and Drug Administration (FDA). Approved agent and continue to be one of the major source of inspiration for drug discovery [10&11].

Natural products may be extracted from plants, a crude (untreated) extract from any one of these source typically contains novel, structurally diverse chemical compounds [1, 12 &13].

Essential oil of chamomile was shown to be a potential antiviral agent against herpes simplex virus type 2 (HSV-2) in vitro P-Potential risks include interference with warfarin and infant botulism in very young children[14].

The present work focus on studying the phytochemical constituents of Matricaria (cammomile) flowers with different extracts with reference to brine shrimps.

Materials And Methods

The method described under the heading of brine shrimp lethality assay. The assay reliability correlates with KB cell toxicity assay. The presence of cytotoxic natural products may be detected during the fractionation and isolation of natural product [15&16].

Materials.

Brine shrimp eggs (*Artemia Sauna*), sea salt, dried yeast, 96-well microplates, DMSO, pasteur pipette, binocular microscope (10.30x), methanol.

Results

Table 1. Extraction of matricaria (chamomilla) flowers with water

| 2 | | 4 | | 5 | 6 | | | | | | | |
|------------|------------------|-------------|-----------------|----------------|-------------|------------|-------|-------|-------|------|--------|--------|
| Con. mg/ml | Mortality mean % | Log con+1 x | Empirical prop. | Expected Prop. | Work. Prop. | Wt. coeff. | w | w.x | Wy | Wx2 | Wxy | Wy2 |
| 1000 | 40 | 4 | 4.75 | 4.75 | 4.75 | 0.627 | 6.7 | 25.08 | 29.78 | 100. | 119.12 | 141.46 |
| 100 | 40 | 3 | 4.75 | 4.40 | 4.78 | 0.558 | 5.57 | 16.74 | 24.55 | 50.2 | 73.65 | 108.2 |
| 10 | 20 | 2 | 4.16 | 4.16 | 0.503 | 10.06 | 20.06 | 20.92 | 20.12 | 2012 | 41.84 | 87.03 |

$Y y^{\circ} + kp$

Kp= percentage mortality

' = $5L88/16.88 = 3.07$

$74.75/16.88 = 4.43$

$V_{50} = 1/0.423 (1/1/sw + 0.164 / x) = 2.364 (0.043+ 2.002+$

$= 0.086\% = 10.0086$

$Ml = M \pm 1.96 \times 0.093 = 3.02 + 0.182 = 3.2 \text{ shift / log}$

Factors $3.02 - 0.182 \ 2.838 \text{ shift! log} = 689$

LC50 = 1036 mg/ml >1000

Brine - shrimp microwell cytotoxicity assay typically consists of the following assay steps:

1. Artificial sea water is prepared by dissolving sea salt in distilled water (40g/lit) supplemented with 6 mg/lit, dried yeast.
2. Brine shrimp eggs (*Artemia sauna*) are hatched in artificial sea water during 48 hours incubation in a warm room (22–29°C).
3. Brine shrimp larvae (nauplii) are collected with a Pasteur pipette and arranged the organisms to one side of the vessel with a light so nauplii are separated from the eggs by pipetting them 2-3 times in small beakers containing sea water.
4. The test sample 20 mg of crude extracts or 4 mg for pure compound) is made up to 1ml in artificial sea water (water insoluble compounds or extracts can be dissolved in 5 ml DM50 prior to adding sea water).
5. Serial dilutions are made in wells of 96-well in triplicates in 100 P1 sea water (Figure 1) and Table 1
6. A suspension of nauplii containing 10-15 brine shrimp larvae (100 ml) are added to each well with the help of a Pasteur pipette and the covered microwell plate incubated at 22-29°C for 24 hours.
7. The plates are then examined under a binocular microscope (12.5x) and the number of dead (non-mobile) nauplii in each well counted.
8. 100 p1 methanol is then added to microwell and after 15 min the total number of nauplii in each well is counted.
9. LC50 values are then calculated by using prop analysis.

Table 2. bioautography method

| | |
|--|----|
| KH ₂ PO | 7g |
| Na ₂ HPO ₄ 2H ₂ O | 3g |
| KNO ₃ | 1g |
| MgSO ₄ 7H ₂ O | 1g |
| NaCl | 1g |

1. UV lamp.
2. Test fungi such as *Aspergillus niger*, *Ascochyta pisi*, *Bally tis cinerea*, *Colletrichum lindemuthlanun*, *Fusarium culrnorum*, *Penicillium expansum*, *Glomerella cingulato*, *Cladosporium clfcumel'inum+* (Caution)
3. Organic solvents S. TLC tanks.
4. Autoclave.
5. Glucose.
6. Incubator
7. Hot air blower.
8. Test sample (crude extract, pure natural product or synthetic compound the chemicals applied were given in Table 2).

Table 3. Antimicrobial activities of water extract

| <i>A spergillus niger</i> | <i>B. subtilis</i> | <i>E. coli</i> | <i>P</i> | <i>C</i> | <i>An</i> | |
|---------------------------|--------------------|----------------|----------|----------|-----------|-----------|
| 20 | 14 | 17 | 14 | 18 | 14 | Chamomile |

Antimicrobial activity were expressed as inhibition diameter zone in millimeters (mm) N.A (no activity) 4mm *B = Bacillus subtilis* ATCC 6633 *P = Pseudomonas aureginosa* ATCC27953 staph staphylococcus Aureus ATCC29213. *E. coli = Ecoh esherchia coli* ATCC25922. *C = Candida albicans* NRRL Y477 and *An A spergillus niger* NRRL.3

Toxicity Assay

Report

Since the RF value of the spot A is identical with that of the spot C (containing caffeine) hence the given sample solution contains caffeine.

Thin layer chromatography for Matricaria

Layer: silica gel

Solvent system: Benzene and ethanol in the ratio of 80:20 respectively.

Detection: By iodine in chloroform 1%.

Sample solution

Small amount of powdered drug is moistened with calcium hydroxide and distilled water to form a smooth paste and then extracted with chloroform. The chloroform layer is separated and evaporated to dryness on a water bath the residue is dissolved in 2 ml of methanol .0.1 ml is spotted by using capillary tube.

Detection of Compounds on TLC

TLC plates were viewed under UV light at 254 and 366 nm for fluorescence or quenching spots. Then sprayed with concentrated H₂SO₄.

R₁ values were calculated as follows:

$$R_1 \text{ value} = \frac{\text{Distance moved by the solute}}{\text{Distance moved by the solvent}}$$

Preparation of thin layer chromatography plates T

The coating materials were usually applied as aqueous slurries. Slurry was made by mixing 30g of silica gel G with 60 mL of distilled water in a motor until it was of uniform consistency and free of air bubbles. The slurry was spread using a dragendroff's reagent spreader at 0.25mm thick layer on five glass plates (20x20 cm)

The coated plates were dried at room temperature, then placed vertically in an oven and activated by heating to 10 °C for 30 minutes.

Column chromatography (CC)

Column chromatography was performed on a glass column packed with silica gel. The extracts were chromatographed after being absorbed onto a small amount of packing material or applied to the top of column by pipette.

Discussion

Phytochemical screening of *Matricaria chamomile* indicated the presence of secondary metabolites give positive result for Alkaloids, Flavonoids, Anthraquinones, Steroids, Tannins, Saponins and Glycosides as expressed in Table 1.

Anti-bacterial activity

The ethyl acetate extract is best solvent gave positive result for all types of bacteria, especially *staphylococcus aureus* as shown in Table 3.

Thin layer Chromatography

The chloroform and ethyl acetate extracts gave the best compound separation for *Matricaria chamomile*. Concentrated ethanol extract isolated three flavonoids compounds (Figure 2).

Spectral data

The maximum absorption for three compounds at peak with RF (Figure 3):

- 0.333 compound A

- 0.305 compound B

- 0.324 compound C

The present study focused on the larvicidal as well as the *Matricaria chamomilla* activity of crude plant extract, comparatively more effective than other mosquito larvae of *Matricaria chamomilla*. In a study of Arivoli and coworkers [17 & 18] had reported that larvicidal property of this plant in ethyl acetate extract against *Brine Shrimp* larvae with an LC_{50} value of 1.63 g/L. The comparison of safety two methods the mosquito larvae was found to be significantly effective method to detect the *Matricaria Toxicity* as a cheap and available method.

Conclusion

The phytochemical screening of *Matricaria chamomile* flowers reflect the presence of flavonoids glycosides, tannins, anthraquinones, triterpene and sterols, saponins and alkaloids. Toxicity was carried out with *Brine Shrimp*, in comparison of safety two methods the mosquito larvae was found to be significantly effective method to detect the *Matricaria Toxicity* as a cheap and available method.

Declarations

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Competing of interest

The authors declare that they have no competing interests

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Figures

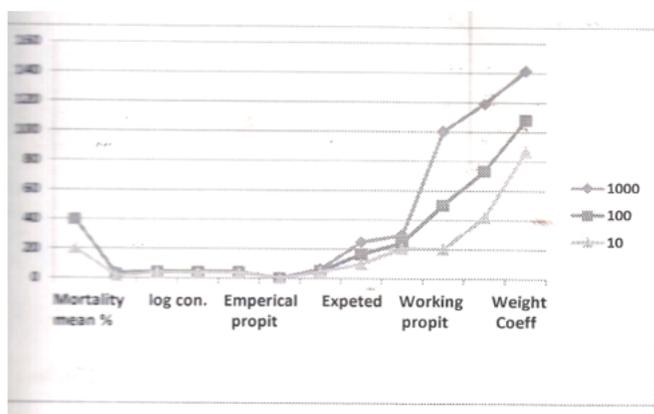


Figure 1

The effect of water extraction with different concentration on matricaria (chamomilla) flowers

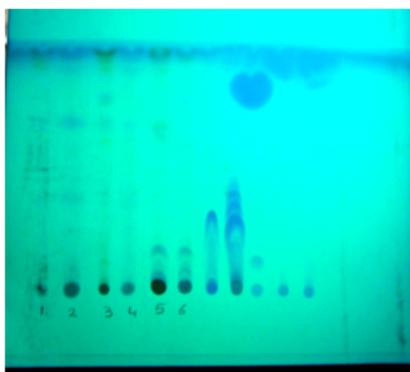


Figure 2

Thin layer chromatography plates for (A) and (B) matricaria ethyl acetate extraction with different separated fraction from silica gel and eluted with different polar solvents system by increasing the methanol percentage in (water methanol system).

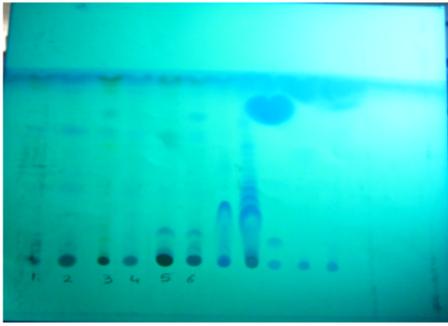


Figure 3

Column chromatography for (A) and (B) matricaria ethyl acetate extraction with different separated fraction from silica gel and eluted with different polar solvents system by increasing the methanol percentage in (water methanol system)