



## In vivo and in vitro Studies of a Polar Extract of *Helianthus annuus* (Sunflower) Seeds in Treatment of Napkin Dermatitis

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### ABSTRACT

Napkin dermatitis is common disease affected neonates. The disease arises from irritation, bacterial and fungal infection and other skin diseases. The aim of the present study is to extract a polar oil from the seeds of sunflower (*Helianthus annuus*) and investigate its antimicrobial activity, using agar diffusion method against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris* and *Candida albicans*; also, determination of the presence of Zinc in the extract using flame atomic absorption technique and to assess clinical advantage of the extract prepared within selected formula in treating Napkin dermatitis. The results showed distinct interest in terms of effectiveness of the extract on growth inhibition of all microorganisms at different concentrations, Where *Staphylococcus aureus* shown the highest rate followed by *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* at concentrations of 1:1 and 1:2, while *Escherichia coli*, *Proteus vulgaris* and *Candida albicans* expressed sensitivity at 1:1 concentration. Also the extract includes a high percentage of zinc, which is essential in the composition of the skin cells. Therefore, the present study proved useful therapeutic results impressive when used the extract topically to treat pediatric diaper rashes.

**Keywords:** Dermatitis, extract, polar oil, pediatric rashes.

### INTRODUCTION

Napkin dermatitis, also known as *diaper rash* or *nappy rash*, is very common disease in babies, it's an inflammatory disorder characterized by the development of erythema, papules and sometimes vesiculation with scaling affecting the napkin or diaper area of usual infants. It caused by: Irritant contact dermatitis: due to urine and feces that will cause a rash on any skin left in contact for long enough<sup>1</sup>. Sometimes ammonia is formed and burns the skin. Also, infection with bacteria and candida yeasts. Psoriasis and atopic dermatitis can affect the napkin area also<sup>1</sup>. Treatment involve application a non-irritating emollient to the affected areas which could be nonionic cream, fatty cream, a mineral oil or wool fat lotion, zinc and castor oil cream, or another favorite. Dimeticone (Silicone) barrier creams may also be used. Some cases required topical steroid and/or antifungal cream<sup>2</sup>.

The use of higher plants and their extracts to treat infections is an ancient practice. Traditional medicinal practice has been known for centuries in many parts of the world. These medicinal plants considered as a rich source of antimicrobial agents and many effective medications<sup>3</sup>. During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics has led to the search of new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures which could overcome the above disadvantages<sup>4</sup>.

*Helianthus annuus*, contains an oleic acid, alkaloid, tannins, fixed oils and simple phenolic compounds. The seeds have medicinal importance as diuretic, expectorant; also they are used for throat and lung infections, cancer and many other diseases. *Helianthus annuus*, possess antimicrobial activity against many bacteria<sup>5</sup>.

The aim of the present study is to extract and investigate the *in vitro* antimicrobial activity of a polar *H.annuus* seeds extract against several pathogenic microorganisms which cause napkin dermatitis; as well as, determination of the presence of zinc in the extract. Furthermore, Clinical testing topically of a mixture containing the extract to treat babies suffering from napkin dermatitis was also done.

### MATERIALS AND METHODS

#### Plant material

The seeds of *Helianthus annuus* used were purchased from local herbal shop in Baghdad. Hexane from Fluka Company, Germany, Starch and gentian violet are manufactured in Iraq.

#### Methods

Extraction, 30 grams of fine powder of *H. annuus* seeds core were soaked in 300 ml of hexane for 24 hours, the extract was filtered and the solvent was evaporated using rotary evaporator, Buchi, Rotavapor.R205, Germany<sup>6</sup>. Total zinc in the oily extract was determined by flame atomic absorption spectrometry technique using AA990 PG, UK apparatus.

### Clinical testing of extract

The oily extract was only applied topically in the treatment of diaper rash for several cases. Additionally, the oily extract was mixed with starch to form a paste that is clinically applicable, more over a selected formula was chosen for subsequent topical studies which was composed of Gentian violet (2.5 ml), Extracted oil (5 ml) and Starch (300 mg) that were well mixed and applied on the affected region two times daily for three days for 20 Babies ages ranging from (6 months – 2 years old).

### In vitro determination of antimicrobial activity

#### Microorganism strain

*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Proteus vulgaris* and *Candida albicans* were used as test microorganisms.

#### Antimicrobial assay

For the antimicrobial tests, hexane extract was dissolved in dimethyl sulfoxide (DMSO) aiming to obtain a final concentration of 1:1, 1:2, 1:3 (v/v). Agar diffusion method was used to determine antimicrobial activity, according to NCCLS criteria<sup>7,8</sup>.

Sterile filter paper discs were prepared to a diameter of 6 mm and sterilized in oven (at 170 C for 2 hrs). The oils were sterilized by passing through 0.22 mm pore size membrane filters.

The culture medium was inoculated with one of tested microorganisms suspended in nutrient broth. Discs were soaked with 20 micro liter oil and placed on the inoculated agar. Solvent was used as negative control. Standard antibiotics Erythromycin (15 microgram/disc), Cephalixin (30 microgram/disc), Tetracycline (30 microgram /disc) and Clindamycin (2 microgram /disc) were used as positive controls. They were incubated at 37°C for 24 hours. At the end of incubation period, the inhibition zones (The areas with no bacterial growth) diameters were measured in millimeters.

### RESULTS

The results showed good effectiveness of the extracted oil treating numerous cases of rash, but at the same time and to get the best results we have been focusing on formula composed of *Helianthus annuus* seeds oil, gentian violet and starch. The clinical therapeutic effect of the prepared formula showed in figure (1) for 12 months aged child treated with that formula.

The effect could be explained by the presence of oil extract, which have antimicrobial activity against many microbes that causes napkin dermatitis beside the presence of zinc element which have skin protection activity. The zinc content in the oily extract was detected by using flame atomic absorption spectroscopy technique, and the level was 16 micro gram/milliliter in the tested oily extract sample.

The antimicrobial activity of *Helianthus annuus* seeds extract (oil) at concentration of 1:1, 1:2, and 1:3 was investigated and the results are presented in table (1). *Staphylococcus aureus* was the most sensitive bacteria; the inhibition zone was 22 mm and 13 mm at 1:1 and 1:2 concentrations respectively. Followed by *Staphylococcus epidermidis* was sensitive at both concentration and the inhibition zone was 10 mm and 8 mm. Gram negative bacteria was less sensitive, the most sensitive gram negative bacteria was *Pseudomonas aeruginosa*, it showed inhibition zone of 11mm and 7 mm at concentrations of 1:1 and 1:2 respectively. Whereas *Escherichia coli* and *Proteus vulgaris* was sensitive to 1:1 concentration only and the inhibition zones were 7 mm and 8 mm respectively. *Candida albicans* showed sensitivity to the extract at 1:1 concentration, with an inhibition zone was 8 mm. No effect was obtained at 1:3 concentrations for all microorganisms. All bacteria was resistant to all antibiotic discs were used.



A - Before treatment



B - After topical application twice daily for 3 days.

**Figure 1:** An 8-month-old boy with napkin dermatitis

**Table 1:** Antimicrobial activities of hexane extract of *Helianthus annuus* seeds.

Tested bacteria	Inhibition zone diameter (mm)		
	Concentration (v/v)		
	1 : 1	1 : 2	1 : 3
<i>Staphylococcus aureus</i>	22	13	-
<i>Staphylococcus epidermidis</i>	10	8	-
<i>Pseudomonas aeruginosa</i>	11	7	-
<i>Escherichia coli</i>	7	-	-
<i>Proteus vulgaris</i>	8	-	-
<i>Candida albicans</i>	8	-	-

## DISCUSSION

Our study demonstrated efficacy of extract of *Helianthus annuus* seeds extract in the topical treatment of napkin dermatitis. In addition, the results proved the effectiveness of the oil in-vitro in the elimination of many microorganisms that always develops itself in drug resistance. The problem of microbial resistance is growing and the outlook for the use of anti-microbial drugs in the future is still uncertain. Therefore, movement must be taken to reduce this problem, for example to control the use of antibiotic and to support development researches towards better understanding the genetic mechanisms of resistance and to continue studies to develop new drugs, either synthetic or natural. Our results agree with the findings of Darmstadt GL, et al that approved the usefulness of sunflower oily extract<sup>9</sup>, however, what distinguishes our study is the extensive antimicrobial studies and the zinc contents of the extract besides optimizing an effective formula make an effort in treating the disease. Plants are important source of potentially useful compounds of characteristic structures for the development of new chemotherapeutic agents. The first step towards this goal is the invitro antimicrobial activity assay<sup>10,11</sup>. The antimicrobial activities of plants have been investigated by a number of researchers worldwide. The effect of oils on bacteria through ability to increase the microbial cytoplasm membrane permeability, probably because their capability of dissolving into the phospholipid bilayer alighting between the fatty acids chains and caused a distortion of the membrane physical structure<sup>12,13</sup>.

According to the antimicrobial assay done for screening purpose, all tested microorganisms were susceptible to the oil. Although Crystal violet inhibited the growth of many bacteria, it has little effects on several types of microorganisms<sup>14</sup>. Association of *Helianthus annuus* seeds oil with gentian and starch to treat napkin dermatitis gave impressive clinical results in the treatment of napkin rash. And where the tests are conducted on different types of bacteria, as well as the deadly impact of fungi, which supports the idea that the effective role of the used mixture is attributed mainly to the oil extract of seeds of the plant under discussion.

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