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Antitumor Effect of Methanolic Extract of *Echinacea purpureas* leaves and Determination of Anti-microbial activity of plant extract *in vitro*

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ABSTRACT

The modern-day study objectives at assessing the in-vitro usage of extracts of *Echinacea* to evaluate their inhibitory effect on pathogenic microorganisms (7 species) were isolated from different environments The methanolic crude extract of leaves of *Echinacea purpurea* were prepared using soxhlet apparatus. These extracts were examined to inhibited microbial strains like *Pseudomonas aeruginosa, S. aureus, Klebsiella pneumonia, E. coli, Streptococcus epidermidis, Aspergillus niger*, and *C. albicans* using the disc diffusion method. Methanol extract is more effective against resistance zone 14 mm against streptococcus. Against the MCF7 cell line, this percentage was increased after 48 hours. To consider the cytotoxic properties of herbal extract alongside human breast cancer (MCF7) cell lines with a concentration range of *Echinacea purpurea* extracts (6.25,12.5, 25,50,100,400 g/ml). With the maximum percentage increase resistance (97%) observed in treatment with 400mg / ml against the MCF7 cell line. The lowest ratios were discussed with MCF7 having a permanent cure (20%) at 12.5 mg/ml. Finally, GC mass analysis was performed for crude extracts to identify the most active chemical compounds of *Echinacea purpurea* extract, resulting in the existence of 26 biologically dynamic composites, including nine composites with antioxidant and anticancer activity.

Key words : *Antioxidant, Gas chromatography & Mass spectrometry, MCF7 cell line, Echinacea purpureaa, Antimicrobial.*

Introduction

Echinacea purpurea is used as a medicinal plant, in the northern United States concerning USA, *Echinacea purpurea* is often administered as an active ingredient to treat the common cold. The plant is very versatile. When given in high doses in a short period, it boosts the immune system, which aims to combat wound infections as well as viral infections (Hudson, 2011). This square contains eleven types of herbaceous yet permanent flowering plants. Specifically, *Echinacea purpurea* is normally distributed throughout the United States, Canada, then among Europe, and thoroughly inside Germany. (Sharifi Rad *et al.*, 2017).

This herbal medicine has a bactericidal effect and has anti-inflammatory characterization, which has benefits of fighting respiratory infection, *Echinacea* also has the function of working alongside viruses, the durability of their action, including legion cells, and maintaining durability by using a nominal length in infected cells, while the animal is much less active on the intracellular side (Hudson, 2011).

The immunomodulatory properties of *Echinacea* were taken out by *in vitro* and *in vivo* alkaloids, which were significant mechanisms of the plant, in accumulation, kefic acid originates in a roundabout species of *Echinacea* and is practical for standardiza-

tion and superiority mechanism of herbal extracts. Polysaccharides show a significant part in the anti-inflammatory consequence of *Echinacea* groundings (Laasonen *et al.*, 2020).

Qq Biofilm discusses to a numeral of compound microbial neighborhoods that are extraordinarily resistant to dark antimicrobials. The shape of the biofilms in the antibiotics beneath the abiotic surfaces is related to a large number of illnesses, including the number of deaths in hospitalized patients.

New alternatives of controlling infections depend on the medicinal effects of medical interline in gathering their antimicrobial properties. Bacterial biofilms are corporations of bacteria, surrounded by a self-generating matrix, growing concerning non-living and then living organization surfaces (Sanchez *et al.*, 2016). Biofilm is considered an emergency virality factor, so recurrent causes under durability employ persistent infections; (Grant, 2013). Extracellular averages (Sun *et al.*, 2013) are safe with the resource because they contain about 75% of the bacterial biofilm contractile, as are intestinal infections (Vasudevan, 2014).

The physical intent to uphold the biofilm ban is to adapt to the specific type of bacterial and fungal infections used, including touching prevention, but the pharmacological development of expectorant tablets is currently widely exciting (Namasivayam *et al.*, 2013).

Echinacea is reflected on consideration on some type about the shut popular Deputationist ancient of remedy upon cancer sufferers (Chicca *et al.*, 2007), its utilization is hourly a loosely desire concerning the alone patient (Dy *et al.*, 2004).

Echinacea extracts have been suggested as an adjunct to cancer chemotherapy. In several studies, it has been reported that Echinacea purpurea extract protects non-cancer cells from apoptosis. Experimentations through water-soluble extracts from the origins of *E. purpurea* have suggested the increase of controlled breast cancer cells. (Goey A, 2013).

The polysaccharide portion sequestered from *E. purpurea* (EPS-EPO VIIa) was shown to condense chemotherapy-induced leukopenia (Melchart *et al.*, 2002).

The objective of this study is to detect whether *E. purpurea* extract is indicated for its various actions (anti-biofilm, anti-pathogenic microorganism, and antitumor effect).

Materials and Methods

Microorganisms in this study

Many currently known species (seven isolates) (*Pseudomonas aeruginosa, S. aureus, Klebsiella pneumoniae, E. coli*, *St. epidermis, Aspergillus niger*, and *Candida albicans* have been identified using conventional microbial methods such as Gram stain reaction and 2 microbial methods. Collected from Al-Khindi Teaching Hospital, Baghdad.

Preparation of Plant Extracts

To formulate the essence, the aerial portions of *E. perpurea* have been initially placed in the air and then fully dried out in the shade. The aerial parts have been fully powdered through a mincer and placed in glass jars. Extraction was carried out at room temperature for up to 12 h. The bury leaves of Echinacea are mixed with the following technology: 1g bury leaves are exposed to dust after 250ml, using 95% methanol at 60 ° C for 3-hours using a soxlite extraction device. Store in a dark place at –80 C for a longer period or sterilize using a 0.20 mm membrane-like filter.

Cell line growth and cytotoxicity assay

The colorimetric smartphone viability MTT test is the oldest incompatibility with the anticancer potential of methanolic echinacea extract, so this technique was once used using About the Road (Freshney, 2012). First, one hundred µL/ well (106 cell/mL) of RD cells were cultured in a 96-well art subculture plate.

Extracts of different concentrations (6.25,12.5, 25,50,100,400) g / ml were administered after thinking about the cytotoxic effect between fight after cellular sequence (MCF7) under the WRL68 common phone line (MCF7). Then, briefly, in a 96-well plate, a 50 supernatant was infused with a 50 µl response combination containing tetrazolium salt, which was concentrated to pharma zone in the occurrence of LDH. The bowl was incubated for 30 min. Secure after light before adding 50 µl stop solution D DMSO (dimethyl sulfoxide) is brought between each well in simulation, with absorption read at 490 nm with absorption at 690 nm to eliminate circumstantial. Vehicle preserved cells has been lubricated with lysine buffer and used as supreme LDH release.

Finally, the absorption was moderate, as each consumes at 620 nm in the ELISA reader. Although the dead cells do not contain the bear, only the successful cells between the cells receive the contaminant.

Live cells are considered in simulation in terms of the percentage on probability and then the consequences with the inhibition ratio formula:

$$GI\% = \frac{(\text{O.D of control wells–O.D. of test wells})}{\text{O.D. of control wells}} \times 100 \quad (1)$$

***In vitro* antibacterial activity of *Echinacea* extract**

Well-diffusion methods

The agar well diffusion method was used to detect the antibacterial movement of *Echinacea* shroud on the nutrient agar, and then 50 µL of the corresponding microbial inoculum was taken using a micropipette to provide an equal turf of the cells and loaded evenly over the agar plates. The agar plates were vaccinated with the matching microorganisms by onslaught the complete appearance of the plate by revolving the petri plates three times at about 60 after each application. Finally, it was wiped around the edge of the agar surface (Attai *et al.,* 1987)

Gas Chromatography and Mass Spectrometry (GC-MS)

For Gas Chromatography-Mass Spectrometry examination, a high-temperature column (inert cap 1MS; 30 m × 0.25 mm id × 0.25 m film thinness) has been procured from Agilent Technologies (SHIMADZU, Japan). Injector and detector temperatures have been set at 280 C, though the original column temperature was fixed at 100 C. First, 5ìl was injected into the sample column and after 1 min was applied via split (1:10) method and the oven temperature were related to 225 °C with a ramp rate of 12.5 °C/ min (hold time 4 min). The oven temperature was augmented to 300 °C at a ramp rate of 7.5 °C / min (hold time 5 min). The helium transferor gas has been set to keep up a persistent stream rate of 17.5 ml / min and the mass spectrum was acquired and administered through together Agilent GC-Mass Solution (SHIMADZU, Japan) and Postsoftware. Compounds are determined by associating their mass with the NIST Library search and standard criteria. (Amirav, 2009).

Statistical analysis

Scan results are displayed separately bare equal deviations. As soon as the one-way ANOVA returned to analysis on variation, the differences between mastery were determined because the magnitude was at p and lt; 0.05.

Results and Discussion

Microorganisms

Seven distinct species of pathogenic microorganisms *(Pseudomonas aeruginosa, S. aureus, Klebsiella pneumonia, E.coli, St. epidermidis, Aspergillus niger and C. albicans)* have been identified according to morphological stability, cultural, and Vitek 2 regulatory characteristics.

Cell line growth and cytotoxicity assay

Methanolic extracts on *Echinacea purpurea*, used to be once investigated by way of potential about the utilization on over vitro cytotoxicity within the opposition in imitation of national mamma most cancers (MCF7, WRL68). The cytotoxic responsibility for methanol sources durability showed dose afterward time-dependent inhibitory effect, the penalties are represented in Tables 1 and 2, Precise absorptions ((6.25,12.5, 25,50,100,400) µg / ml of methanol source were practical and growth resistance percentage was intended 24 and 48 hours after incubation. As per the results, extreme development inhibition (20%) has been detected through treatment through 12.5 mg/ml against MCF7. Additionally, such stability percentages were accelerated after 24 h. In addition, it was once demonstrated internally (Figure 1) With the aid of calculating IC50, proving significantly the most dynamic cytotoxic exercise including IC50 Honor 18.68 concerning the Echinacea MCF7 cell line, however, the IC50 property was recorded concerning longevity 298.7. WR68L (common cell line strata) (Figure 2).

Additionally,, It was once performed inside (Figure 1) with the aid of calculating the IC50, the Echinacea cast-off proven considerably the most dynamic cytotoxic exercise including IC50 honor 18.68 regarding MCF7 cell line Whereas, the IC50 virtue longevity was recorded 298.7 µg/ml concerning the WR68L (normal cell line strata) (Figure 2)

The outcomes within (Yu-Ling Tsa i *et al.,* 2012) longevity confirm enormous inhibition of proliferation in *E. purpurea* bloom, which eliminates genital colon cancer cells by the organization on apoptosis, as well as warning of up-and-coming anti-tumor

activity. Extensive cite durability *E. purpurea* bottom yet *E purpurea* slip BT-549 is compatible with chicoric acid reduction size, stability is a living element above Echinacea so size specifically *E. purpurea*, (Driggins *et al.,* 2017).

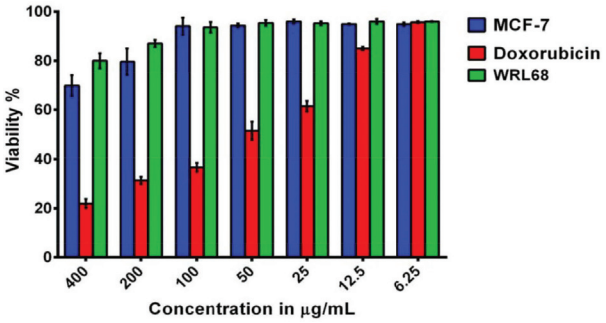


Fig. 1. The effect of methanolic extract of *Echinacea* leaves on MCF7 cell line

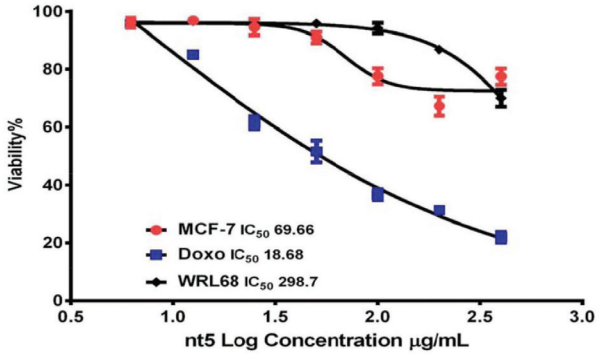


Fig. 2. The Ic50 of methanolic extract of ***Echinacea*** leaves on MCF7 cell line

Antimicrobial Activity of crude extracts of Echinacea purpurea

Results of the Table 1 Figure 3, 4 longevity excessive considerable particular in gross extracts touching *Echinacea purpurea* above numerous kinds relating to microorganisms examine along with mechanism, These extracts appearance antibacterial activity agreeing to *Streptococcus epidermis* cooperatively together with an inhibition region used to be 14 mm, yet it had the bad result antibacterial activity amongst imitation allowing to *Pseudomonas* (10 mm), *E. coli* (13 mm).

This result accepts including a quantity respecting have been evolved abroad below inspect or below prove the traditionally referred after antibacterial recreation concerning the plant life belonging of conformity over the crew *Echinaceae* then their preparations. (Rizzello *et al.,* 2013; Fatima Amer *et al.,* 2019)

Sharma *et al.* (2010) examined the bactericidal challenge for ethanol recovery (65%), with the ethereal components collected later but the sources about *E. purpurea,* at concentrations of (100 t0 20) ml, becoming dry in simulation. Respiratory Infections: *L. pneumophila, Streptococcus pyogenes, Mycobacterium smegmatis*, strains with excellent care and sensitivity to consistency.

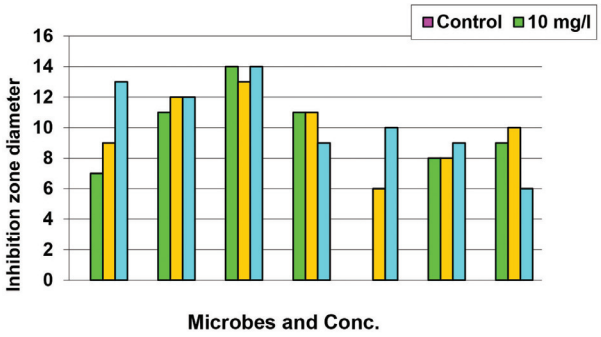


Fig. 3. Antimicrobial Action of crude extracts of *Echinacea purpurea* as presented by inhibition zone diameter (mm). against (*E. coli , Klebsiella, St. epidermis, S. aureus, Pseudomonas, Aspergillus niger, C. albicans)*

Table 1. Antimicrobial Action of crude extracts of *Echinacea purpureaas* represented by inhibition zone diameter (mm)

Microbes	Control	10 mg/l	20mg/l	30mg/l	LSD value
E. coli	0	7	9	13	2.88 *
Klebsiella ,	0	11	12	12	2.04 *
St. epidermis	0	14	13	14	3.52 *
S. aureus	0	11	11	9	2.19 *
Pseudomonas	0	0	6	10	3.08 *
Aspergillus niger	0	8	8	9	2.17 *
C. albicans	0	9	10	6	2.52 *
LSD value	—	3.61 *	3.09 *	3.73 *	—-

* (P<0.05).

In addition, the guilty microorganism because pneumonia, *L. pneumophila* operate continue to be additionally inhibited through *E. purpurea* (Hudson, 2011).

The lively elements along with the antimicrobial properties of emulation in imitation of pores and skin pathogens are contemporary in Echinacea spp. plants, subsequent so magnitude was one-time endurance carefully chosen via proficiency above lifetime Rehman *et al.*, (2012). *Saccharomyces cerevisiae* after *Candida albicans* has been shown to significantly prevent the enlargement of *Saccharomyces cerevisiae* due to its isolation from Purperia, although no bans have been imposed due to *Aspergillus niger* (Stojicevic *et al.*, 2009).

An extensive variability of compounds were retained in dependability, with different types of slight metabolites impassive, followed by *E. purpurea* extract. These are the 3 major businesses related to polysaccharides, alkaloids but also kefir sour style products, low metabolites in inter recov-

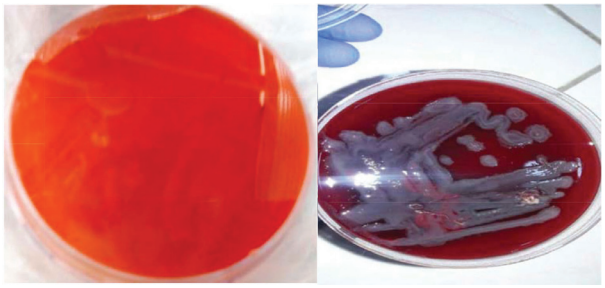
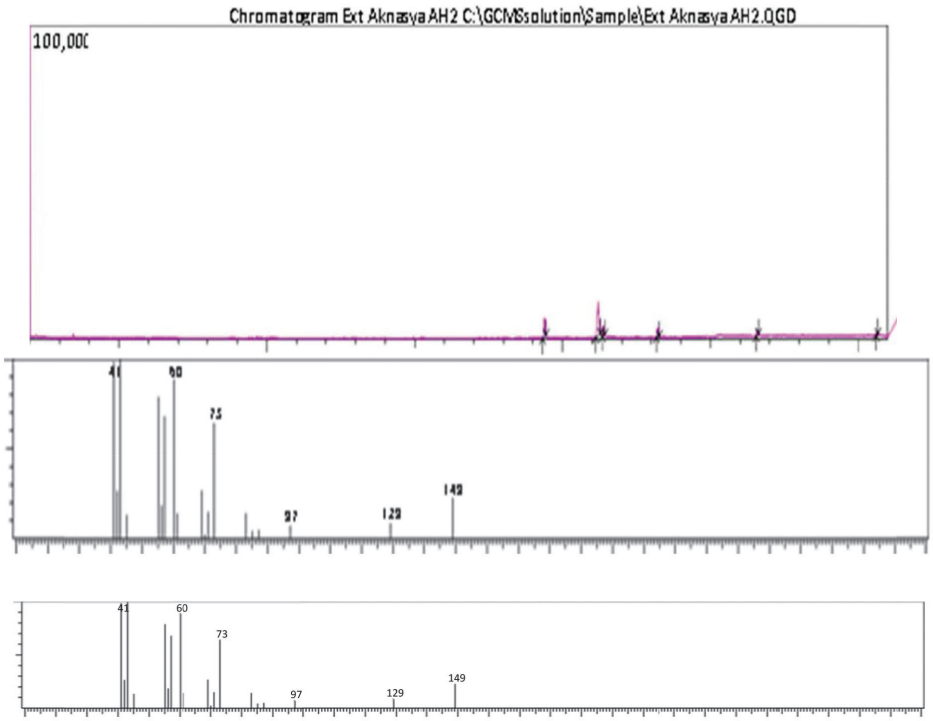


Fig. 3. Antibavteial activity of crude plant extract aginst Klebsiella,

Table 3. Major Phyto-components acquired through the GC/MS Study of *Echinacea* spp leaves extract

No.	Name of compound	RT	Area %
1	Hexadecanoic acid	19.378	19.70
2	Palmitic acid	19.378	19.70
3	Pentadecanecarboxylic acid	19.378	19.70
4	Nonadecanoic acid	19.378	19.70
4	4-Methyloctanoic acid	19.378	19.70
5	Tridecanoic acid	19.378	19.70
6	1-Fluorooctane	19.378	19.70
7	9-Octadecenoic acid	21.175	57.15
8	1,3-Propanediol	21.175	0.24
9	9-Hexadecenoic acid	21.175	0.23
10	Pentadecen-1-ol	21.175	0.82
11	Heptanoic acid	21.350	0.86
12	Enanthic acid	21.350	0.63
13	n-Heptanoic acid	21.350	0.91
	e:Isoamyl nitrite	21.350	1.49
	Nitrous acid, 3-methylbutyl ester, Isopentyl alcohol, nitrite	42.216	1.33
14	Aspiral, Isopentyl nitrite, Nitramyl, Vaporole	42.743	0.85
15	Pentanoic acid, 4-methyl-	42.87	40.21
16	Valeric acid, 4-methyl	42.200	0.04
17	3-Hexanol		
18	Ethylpropylcarbinol r		
19	2-Butene, 1-propoxy	44.953	0.57
20	Pentanal, oxime ,ester	45.483	1.87
21	Valeraldehyde, oxime	45.757	0.60
22	Butanoic acid, 3,3-dimethyl-	46.575	5.58
23	-Propene, 2-methyl-3-(1-methylethoxy)-	46.514	0.20
24	Propane-1,3-diol, 2-methyl-	26.575	0.20
	Oxalic acid, allyl hexyl ester	26.575	5.58
	Benzene, 1,2-bis(hexyloxy)-4-nitro-	26.575	5.58
	-ethyl-3-propyCompName:Butanenitrile, 3-methyl- 26.575æ	26.575	5.58
25	Isovaleronitrilel-		
26	æ-Propane,	26.575	5.58
	Isobutyl nitrite	26.575	5.58
	Nitrous acid, 2-methylpropyl ester	26.575	5.58



Gas Chromatography-Mass spectrophotometery chromatogram obtainable the methanolic extract of *Echinacea spp.* leaves

ery, so saving between articles is considered fast (Bauer *et al.,* 1988) Table 3.

Purification utilizing skill about chloroform eliminate overse about under wretched moreover beginning half alkamides consisting of 2-methyl butyl amide moieties yet isobutyl amide then nitidanindiisovalerianate concerning collection between accordance about a sequesterpene, 1β-hydroxy-4 (15),5E,10 (14)-germacratriene, the use over with the resource concerning chromatographic approaches (Hohmann *et al.,* 2011). Remote alkamides alongside isobutylamides durability generally contained (2,4-dienoic) gadgets within their companies (Barnes *et al.,* 2005).

Echinacea Square is honored on behalf of its well acreage (Erenler *et al.,* 2015; Yamada *et al.,* 2011). These generally correspond to their chemical elements (Knöss under Wiesner, 2017). Many types of elements are remotely attached to the aerial parts, and then to the plant roots. Showcase Table 2 shows the number of these generally concerned alkaloids, kefir lotose blind, alkaloids, polyphenols, risky toughness compounds, polysaccharides and even less modified structures (Yu *et al.,* 2013) showcase Table 2.

The antifungal job was found near UV light radiation, and the anti-candida job was even more advanced in modern times than *E. purpurea* extracts (Barrett, 2003). Action in the direction of piece meal yeast strains, including *Candida albicans*, were earlier termed by *Saccharomyces cerevisiae* due to n-hexane extracts from *E. purpurea* sources (Bins *et al.,* 2000). (Binns *et al.,* 2000)

Antibacterial recreation between antagonism according to *Pseudomonas aeruginosa , Escherichia coli, Staphylococcus aureus, and Proteus mirabilis* has been hooked above because of a multi-herbal lookup grudging *E. purpurea* lousy extract, also although such back in imitation of keep listed upon to expectation the detected antibacterial consequences were nearly in entire possibility attributable afterward certain over the ingredients, exclude on onion (Binns *et al.,* 2000).

Gas Chromatography-Mass spectrophotometery chromatogram obtainable the methanolic extract of *Echinacea spp.* leaves

Conclusion

During that entire study, we should consider availability atop amongst vitro cytotoxicity results over *Echinacea* leaves addition to assisting in the detection

of many tumor resistant components through GC mass analysis, it is hoped that this plant can be used as an alternative medicine to treat cancer in the future, as opposed to MCF7 cell lines without adaptation to the simulation of detection along with antitumor elements. Further research is needed to be aimed at its usage, especially in the circumstance of scientific breast cancer treatment.

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