BEST: International Journal of Humanities, Arts, Medicine and Sciences (BEST: IJHAMS)
ISSN (P): 2348-0521, ISSN (E): 2454-4728
Vol. 3, Issue 12, Dec 2015, 43-48

IN – VITRO EVALUATION OF ANTIBACTERIAL ACTIVITY OF *SILYBUM MARIANUM* SEEDS OIL, ETHANOLIC EXTRACT AND ANTIBIOTICS AGAINST ISOLATED BACTERIA

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ABSTRACT

The present study aimed to investigate the antibacterial activity of ethanolic extract of *Silybum marianum* seeds, it's oil, Chloromphincol, Rifampin and Methicillin against isolated bacteria, evaluation is done in-vitro by using hole plate method for appearance zone of inhibition. Antimicrobial susceptibility test showed that their were no antibacterial activity of siylmarin extract while the oil showed very mild effect against *Enterbacter ludwigii, Klebsiella pneumonia* and *Pontoea gaviniae.* Chloromphincol showedeffects aginst most the isolated bacteria except *Citrobacter freundii, Enterobacter cloacae* and *Psudomonous aerogenosa*, Rifampin affect only on *Pontoea gaviniae* while Methicillin didn't show any affects .Chloromphenicol *showed intermediate effect on Enterbacter ludwigii, Enterobacter sakazakii, Klebsiella pneumonia, Pantoea agglomeruns, Proteus mirabilis, Salmonella typhomurium,, while Staphylococcus hominins were* Susceptible, most the isolated bacteria were resistance to Rifambin except *Pontoea gaviniae* gave intermediate inhibition zone. in case of Rifampin no effect were recorded.

KEYWORDS: *Silymarin***,** Chloromphincol, Rifampin and Methicillin

INTRODUCTION

Silybum marianum is a wild growing annually herb that grow in many part of the world include the north part of Iraq and some area north Baghdad city **(Rajiha 2012).** The seeds contain 22% oil ((hydrocarbons, sterols, and fatty acids) **Hammouda *et al.*, 1994).** in the Nile region (Delta) and Fayium region near water streams. The plant has been locally cultivated successfully medical purposes and huge amounts of the oil-rich seeds**, (Hassan et al 2003).** Silymarin, obtained from Silybum marianum is used for hepatoprotection (**Parveen *et al.*, 2012**). Its antioxidant, anti-inflammatory and anti-apoptotic effects these properties have implicated this compound as a potential renoprotective agent **(Zeynab 2012).**

Michelin *et al., (*2005), Zuanazzi, and Montanha (2004) pointed the synergistic drug-modifying effect when silymarin and silibinin were combined with antibiotics and as adjuvant, against the different bacterial strains. **Dayanne *et al.,(*2015)**Their results for the antifungal activity of silymarin and silibinin demonstrated a MIC of 1024 µg/mL

Chloramphenicol known as a broad-spectrum antibiotic produced by Streptomyces **(Lin et al., 2005).** Thus, chloramphenicol is being reconsidered as an option for treatment of certain infections in critically ill patients **(Falagas and Kopterides 2007).** Rifampin, is a antibiotic used to treat a number of bacterial infections includes tuberculosis, leprosy. Often it is used along with other antibiotics. Rifampin used to prevent *Haemophilus influenzae* type b and meningococcal disease (The American Society of Health-System Pharmacists.2015). Meticillin was developed by Beecham in 1959, It was previously used to treat infections caused by susceptible Gram-positive bacteria, *Staphylococcus*

aureus (**Graham 2009)**

MATERIAL AND METHODS

Some Gram-positive bacteria and Gram-negative bacteria were used throughout this study which kindly supplied by research laboratory from Department of Basic medical sciences- college of nursing - University of Basrah. *Silybum marianum* seeds were brought from the north of Iraq (mossel), Seeds were grinded using grinder prior to extraction.50 grams each time were defatted in a soxhlet apparatus, using normal hexane (boiling point of 40C°) for 3 hr ,the oil was separated by distillation, 150 ml of absolute ethanol was added to the remainder amount of defatted seed powder and stirrered for 72 hr. by magnetic stirrer at room temperature. After filtration and concentration of the silymarin fraction under vacuum, the yellow residue was dissolved in 20 ml of toluene and evaporated for one hour. 20 ml of diisopropyle ether add to the crystals and refluxed for 1hr. and cooled for 1 hr. The mixture filtrated by vacuum and the remained crystals were collect, dried, weighted and Stored in deep freezing. **(Wallace *et al*., 2003).**

For Antibacterial activity the well agar diffusion method was used to determine the antimicrobial activity of the prepared extracts oil and antibiotics .mixed 0.6 ml of the standardized bacterial stock suspension (108-109) colony forming units per mL with 60mL of sterile nutrient agar thoroughly. Poured 20ml .inoculated nutrient agar into sterile petri dish Left the agar to set and four well 10mm in diameter was made in each of these plates using sterile cork borer NO 8 and then removed agar disc. Filled the entire well with0.1 of extract using micropipette and allowed to diffuse at room temperature for two hours. The plats were then incubated at 37°C for 24 hours. three replicates were then also performed for each extract and antibiotics against each of the test bacteria (Lee *et al*., 2003;Hanna *et al*., 2008) .For other antibiotic (Chloromphincol, Rifampin and Methicillin) use Kirby-Bauer disc diffusion (DD) methods (Woods et al.,1995).A suspension of the colonies with 0.85 per cent normal saline was made, opacity adjusted to 0.5 McFarland and used for performance of the procedure as described previously, after the inoculated plates had dried sufficiently the discs were placed on the medium, gently pressed and plates incubated at 37°C for 24 hours, each zone size was interpreted with reference standards as susceptible, intermediate and resistant.

RESULTS AND DISCUSSIONS

The ethanolic extract from *Silybum marianum* seed and it's oil did not show any bacterial effects against any of the isolated bacteria used the this study (Table 1).

Mukarram Shah *et al.,(*2011)showed that silymarin both from blue and white capitulum's seeds of *S.marianum* has not shown zone of inhibition against fungus.and all the gram-negative bacteria also show resistance to silymarin while Silymarin has been found very active against all gram positive bacteria.

Dayanne *et al., (*2015)indicated the possibility of the usage of silymarin and silibinin as adjuvants in the antibiotic therapy against multidrug resistant bacteria (MDR), being a promising choice against the concerning problem of the antibiotic resistance.

Enterbacter ludwigii, Enterobacter sakazakii, Klebsiella pneumonia, Pantoea agglomeruns, Proteus mirabilis, Salmonella typhomurium,, gave indeterminate(14-16)inhibition zone and *Staphylococcus hominins were* Susceptible *(17)* to Chloramphenicol while the other bacteria were resistance on other hand most the isolated bacteria were resistance to rifambin except *Pontoea gaviniae gave intermediate inhibition zone.* Methicilin showed negative effect on all isolated

bacteria(table 1).

Methicillin, a β-lactam antibiotic, acts by inhibiting penicillin-binding proteins (PBPs) that are involved in the synthesis of peptidoglycan, an essential mesh-like polymer that surrounds the cell. Methicillin resistance in clinical isolates has been reported to arise from expression of a methicillin-hydrolysing β-lactamase and through the expression of an altered form of PBP2 that has a lower penicillin-binding affinity and higher rates of release of the bound drug compared to the normal PBP ,methicillin resistance is affected by the inactivation of genes that affect the autolytic enzyme activities of the cell. Inactivation of the llm gene, coding for a protein of unknown function, converts a homogeneous strain to a heterogeneous phenotype and is associated with increased autolytic activity **(Maki et al., 1994; Montanari et al., 1996; Tschierske et al., 1997).**

Moreover, Resistance to rifampicin (RIF) is a broad subject covering not just the mechanism of clinical resistance, nearly always due to a genetic change in the β subunit of bacterial RNA polymerase (RNAP), but also how studies of resistant polymerases have helped us understand the structure of the enzyme, the intricacies of the transcription process and its role in complex physiological pathways. RIF-resistant (RIF^r) clinical isolates of several different bacterial species, and a single mutation predominates in mycobacteria **(Goldstein, 2014).**

Chloramphenicol binds to the 50S ribosomal subunit and inhibits the peptidyl transferase step in protein synthesis. Resistance to chloramphenicol is generally due to inactivation of the antibiotic by a chloramphenicol acetyltransferase, various enzymes have been described and are coded for by the cat genes found in gramnegative and gram-positive bacteria and usually show little homology **(Traced et al., 1993 ; Kehrenberg et al., 2001).**

Table 1: Diameter of Zone Inhibition of *S. marianum* Extract. *S, marianum* oil Chloramphenicol,. Rifampin and Methicillin against Gram Positive and Gram-Negative Bacteria

NO	Bacteria	Zone of inhibition mm ((Mean ± SE)				
		S. marianum extract	*S. marianum Oil*	C	RA	M
1	*Citrobacter freundii*	-	-	-	-	-
2	*Enterbacter ludwigii*	-	8.200±160	18.566±0.484	-	-
3	*Enterobacter asburiae*	-	-	8.133±0.881	8.600±0.305	-
4	*Enterobacter cloacae*	-	-	-	-	-
5	*Enterobacter sakazakii*	-	-	15.333±0.260	-	-
6	*Klebsiella pneumoniae*	-	8±0.00	15.233±0.284	-	-
7	*Pantoea agglomeruns*	-	-	15.066±0.666	-	-
8	*Pontoea gaviniae*	-	9.340±0.121	13.200±0.115	13.200±0.115	-
9	*Proteus mirabilis*	-	-	19.100±0.100	-	-
10	*Proteus vulgaris*	-	-	14.100±0.208	-	-
11	*Psudomonous aerogenosa*	-	-	-	-	--
12	*Salmonella typhomurium*	-	-	15.400±0.230	-	-
13	*Staphylococcus hominins*	-	-	17.000±0.00	-	-
14	*Staphylococcus saprophytae*	-	-	13.466±0.290	-	-

C=Chloromphincol, RA= Rifampin, M= Methicillin, Resistance =R≤ 13, Susceptible =S≥ 17, indeterminate= >14-16

CONCLUSIONS

The recent study concluded that silymarin extract, silymarin oil, Rifampin and mithicilin have no bacterial activity against studied bacteria, Chloramphenicol showed mild effects on most of the bacteria *Staphylococcus hominins* detected

the more Susceptible to Chloramphenicol.

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