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EVALUATION OF PHYTOCHEMICALS AND BIOLOGICAL ACTIVITY OF *SILYBUM MARIANUM* L.

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ABSTRACT : The goal of this work is the evaluation of phytochemicals and biological activity of *Silybum marianum. S. marianum* L. and seeds of *Chenopodium murale* L. were collected from different sites from Al Anbar, Iraq during the period of May 2017. Total phenolics, tannins, alkaloids, flavonoids and saponins, were determined. The antioxidant activity was measured based on the reduction of DPPH (1,1-diphenyl-2-picrylhydrazyl). Antibacterial activity of the extracts was determined against ten microorganisms using the disc diffusion method as well as the allelopathic potential of the *S. marianum* extract on the germination of *C. murale* was studied. The IC_{50} values of Methanol extract of *S. marianum* extracts had the highest scavenging activity (1.99 mg.ml^{-1}). The radical scavenging activity of the other extracts and standard decreased in the following order: catechol, ethyl acetate, hexane and petroleum ether. In the present studythe *S. marianum* extracts exhibited different inhibitory activities against the tested microorganisms with different degrees, methanol and ethyl acetate extracts of *S. marianum* showed the broad spectrum against the tested microorganisms. The pathogen *Escherichia coli* was the most sensitive bacteria (25 mm), while *Aspergillus fumigatus* and *A. niger* were the most sensitive fungi (25 and 27 mm) in case of methanol extract. The phytotoxicity of *S.marianum* extract swas increased significantly with the increasing extract concentrations. At 25 g.l^{-1} and 20 g.l^{-1} the germination of *C. murale* was reached maximum inhibition (92.45% and 76.84, respectively). In case of *C. murale* radicle growth, the higher concentration (25 g.l^{-1}) was strongly inhibited (97.23%) the *C. murale*. Similarity, the plant extract significantly reduced the plumule length of *C. murale*. The highest concentrations (25 g.l^{-1}) of extract showed strong inhibition (87.29%) of plumulegrowth. *S. marianum* extracts can be used as natural antioxidant, antimicrobial agents pharmaceutical and bio-control of weeds.

Key words : Phytochemical and biological activity, *Silybum marianum.*

INTRODUCTION

The utilization of plant and its products has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic medicine. Medicinal plants are of play vital importance to the healthof individuals and communities. Herbal medicines derived from plant extracts are beingincreasingly utilized in traditional treatments for many human diseases for thousands of years and in many regions of the world [1]. Plants provide abundant resources of antimicrobial compounds and have been used for centuries to inhibit microbial growth[2].

The medicinal value of many plants species reported having pharmacological properties due to the presence of various kinds of phytochemicals including alkaloids, flavonoids, glycosides, saponins, steroids, terpenes and other phenolic compounds which are therefore, should be utilized to against the disease-causing pathogens [3,4]. The screening of the nutritional composition as the relevance of the presence of phytochemical and antioxidative potentials in a wild plant. Antioxidants or inhibitors of oxidation are compounds which retard or inhibit the oxidation and in general prolong the life of the oxidizable matter [5,6].

Silybum marianum (milk thistle, family Asteraceae) is an annual or biennial herb. Its stem is 20 to 150 cm in height while its leaves ranged from 25 to 50 cm lengthy and 12 to 25cm wide. The fruit is hard-skinned achene with brown spots and is 15 to 20 mm lengthy[7].

S. marianum is native to southern Europe,mainly the Mediterranean region, indigenous to Asia, Southern Europe, North America, and Russian Federation. It is naturalized in South and North America, Australia, China, Central Europe [8].

S. marianum is a wild growing annually herb that growsinmanypartsoftheworldincludingthenorth part of Iraq and some area north Baghdad city [9].

The plant is known for its medicinal properties having essential biochemical constituents including many flavonolignans collectively known as silymarin. Silymarin has antioxidant properties and utilized as part of hepatic disorders, including hepatotoxicity secondary to acute and chronic viral hepatitis and mushroom poisoning [10]. The present study was conducted to evaluate phytochemicals and biological activity of *S. marianum*.

MATERIALS ANDMETHODS

Plant Material and Preparation of the Extract

Silybum marianum L. was collected fromdifferent sites from Al Anbar, Iraq during the period of May 2017. The identification of species was done according to Boulos [11]. It was dried at room temperature and grinded into a powder using a blender. Ten gram of dried plant powder was extracted using different solvents (Methanol, hexane, petroleum ether and ethyl acetate) by socking overnight with periodical shacking. The solution was filtered and evaporated to dryness. The dried residue was dissolved in dimethyl sulfoxide (DMSO) and preserved at -20°C for future use[12].

PhytochemicalAnalysis

S. marianum was collected and prepared as previously mentioned. Total phenolics,flavonoids and alkaloids were estimated using spectrophotometric techniques adapted by Harborne [13], Sadasivam and Manickam [14] and Boham and Kocipai-Abyazan [15], respectively. Tannins were determined according to Van-Buren and Robinson [16], while saponin amount was estimated by the method adoptedby Obadoni and Ochuko[17].

Biological Activity

Evaluation of DPPH free radicalscavenging activity

Antioxidant activity was determined by using a stable free radical (1,1-diphenyl-2-picrylhydrazyl) DPPH [18]. Two ml of 0.15 mM DPPH was added to 2 ml of plant extracts in different concentrations (4000, 2000, 1000, 500 and 250 ppm). A control was prepared by adding 2 ml of DPPH to 2 ml solvent. The mixture was kept in dark at 37°C for 30 min. The absorbance was recorded at 517 nm and the IC_{50} was calculated graphically. The antioxidant activity was expressedas:

% Radical scavenging activity = [1– (A sample/A control)] × 100

Antimicrobialbioassay

Antibacterialactivity

Antibacterial activity of the extracts was determined against seven bacterial strains, three gram-positive i.e., *Streptococcus pyogenes, Staphylococcus aureus and Bacillus subtilis* and four gram-negative i.e., *Proteus vulgaris, Klebsiella pneumoniae, Shigella and Escherichia coli* using the disc diffusion method. A sterile paper disc (5 mm in diameter) was socked in the crude extract of the studied plant and then placed over the surface of the inoculated nutrient agar in antibacterial assay [19]. All Petri dishes were incubated at 37°C for 24 hrs. After incubation, the diameter of inhibition zone (cm) was measured for recording the clear zone and compared with the DMSO as a control. Experiments were performed in triplicate and mean inhibitory zone was calculated. The standard antibiotic of ampicillin, clotrimazole and penicillin were used for comparison with the tested plantextracts.

Antifungalactivity

Antifungal activity against three fungal strains (*Aspergillus fumigatus*, *Aspergillus niger* and *Candida albicans*) was determined by the disc diffusion [19]. Filter paper discs (5 mm in diameter) are prepared before use and sterilized in an autoclave for 20-30 min. A sterile paper disc is wetted in the solution of crude extract (100 µl) and then placed over the surface of the inoculated PDA in the antifungal assay described by Culture plates were incubated at 28°Cfor72 hrs. and zones of inhibition

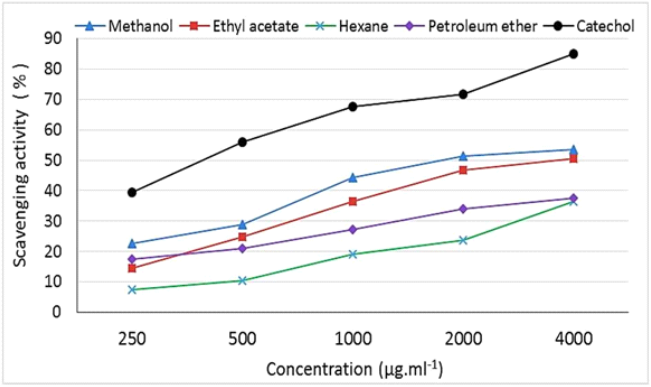

Fig. 1 : % of scavenging activity of *Silybum marianum* extracts and natural antioxidant catechol.

Table 1 : The concentration of the active constituents in mg/g dry weight for the *S. marianum.*

Plantspecies	mg/g Dryweight				
S. marianum	**Tannins**	**Saponins**	**Alkaloids**	**Flavonoids**	**Phenolics**
	11.42±0.65	9.54±0.3	7.33±0.5	9.12±0.52	16.3±1.07

was recorded around the paper disc.

Allelopathicbioassay

The seeds of *Chenopodium murale* were collected from the cultivated land from Al Anbar, Iraq. Seeds were sterilized by 0.3% sodium hypochlorite for 3 minutes, washed several times with distilled water, dried at room temperature for 7 days and reserved in a paper bag until further use [20]. For bioassay assessments, methanol extracts were set up at distinct concentrations (2.5, 5, 10, 20% w/v). The solutions had been filtered via double layers of muslin cloth followed by a Whatman No. 1 filter paper. The pH of the mixtures was adjusted to 7 with 1M HCl, and then mixtures have been kept in a refrigerator at 4 °C till additionally utilize[21].

For germination experiment, two layers of filter paper (Whatman No. 1) were placed in 90 mm diameter sterilized Petri dishes. In each dish, 20 seeds were settled and 10 ml of each plant extract (2.5, 5, 10, 20% w/v) was added. The control treatment was designed with distilled water. Germinated seeds were counted daily starting from the first day of treatment. The design of the

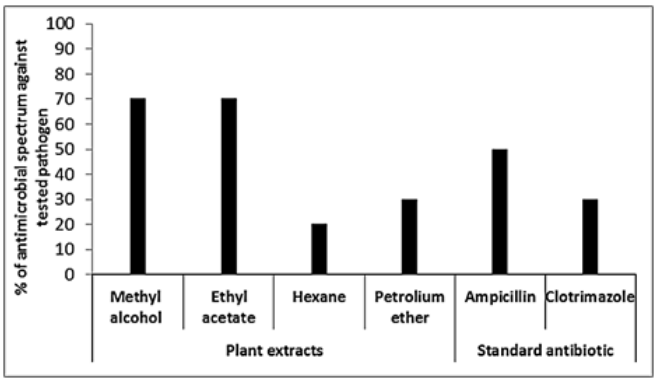

Fig. 4 : % of antimicrobial spectrum of *S. marianum* extracts and standard antibiotic

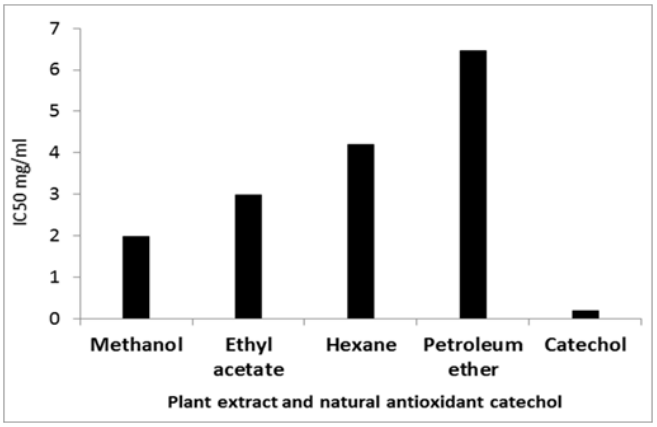

Fig. 2 : IC_{50} values (mg.ml^{-1}) of *Silybum marianum* extracts and natural antioxidant catechol (standard).

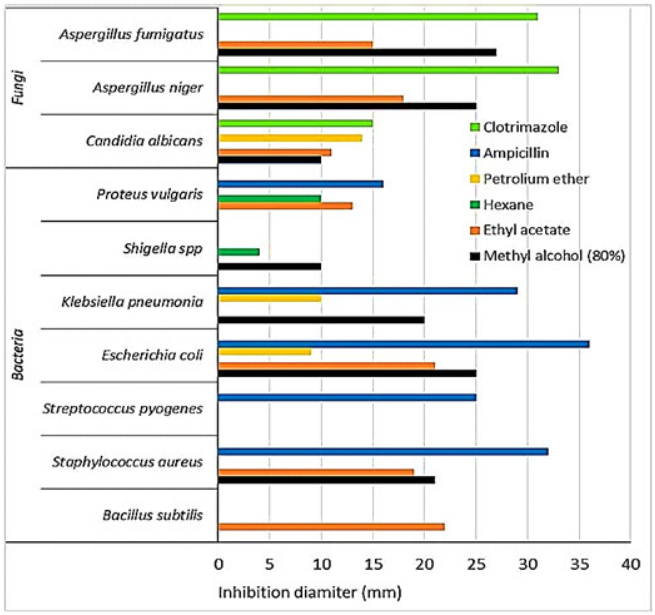

Fig. 3 : Antimicrobial activity of different extract of *S. marianum* and standard antibiotic.

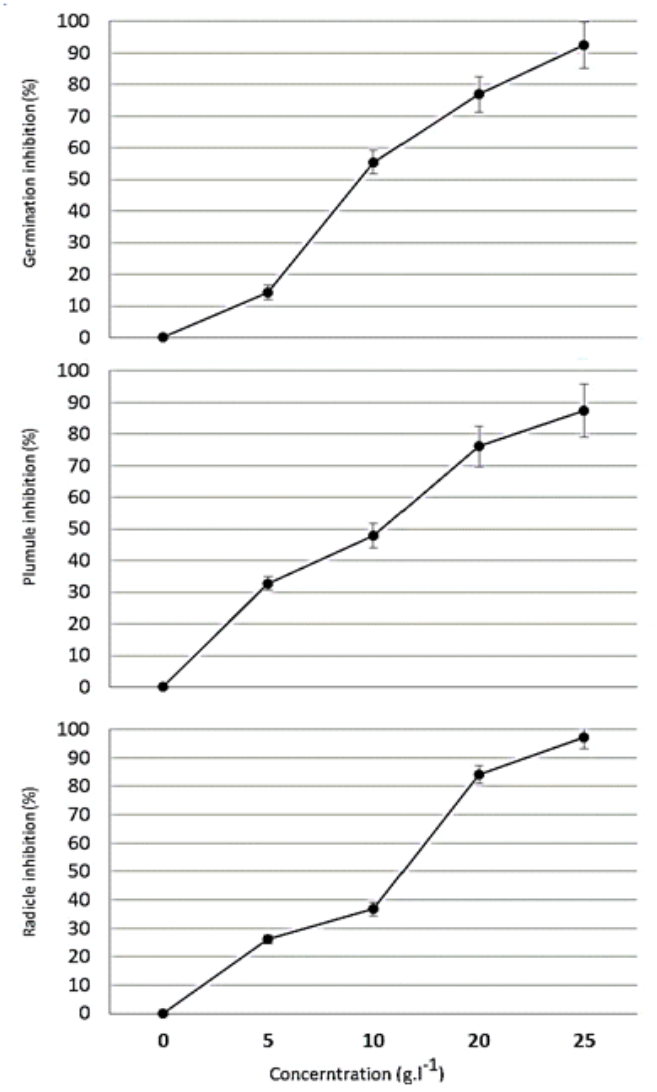

Fig. 5 : Allelopathic effect of different methanol extracts from *S. marianum* aerial parts on the germination and seedling growth of *C. murale* after ten days of treatment.

Table 2 : The inhibitory activity of the plant extract against the tested organisms as demonstrated by diameters of the inhibition zone (mm)*

Test microorganisms	Plant extracts			Standard antibiotic		
	Methyl alcohol	Ethyl acetate	Hexane	Petroleum ether	Ampicillin	Clotrimazole
Gram-positive bacteria						
B. subtilis	-	22	-	-	-	-
S. aureus	21	19	-	-	32	-
S. pyogenes	-	-	-	-	25	-
Gram-negative bacteria						
E. coli	25	21	-	9	36	-
K. pneumonia	20	-	-	10	29	-
Shigella spp	10	-	4	-	-	-
P. vulgaris	-	13	10	-	16	-
Fungi						
C. albicans	10	11	-	14	-	15
A. niger	25	18	-	-	-	33
A. fumigatus	27	15	-	-	-	31

experiment was a randomized complete block with three replicates. The experiment repeated three times and the inhibition percentage was calculated.

The seeds of *C. murale* were germinated in the dark at room temperature for 48 hrs. Twenty germinated seeds were placed in Petri dishes lined with two layers of filter paper (Whatman No. 1) and 10 ml of different extracts (2.5, 5, 10, 20% w/v) were added. Moreover, a control treatment was designed with distilled water. The design of the experiment was a randomized complete block with three replicates. The experiment repeated twice, the radicle and plumule lengths of seedlings were measured on the tenth day and growth inhibition for radicle and plumule lengths werecalculated.

RESULTS AND DISCUSSION

PhytochemicalConstituents

Phytochemicals are playing a vital role for the treatment of different types of diseases and still are used in, both traditional and modern system of medication. The phytochemical analysis of the aerial parts of *S. marianum* indicated that the plant is rich in secondary compounds. Theresults indicated that the *S. marianum* exhibited the highest content of tannins and phenolics (11.42±0.65 and 16.3±1.07 $mg.g^{-1}$, respectively), followed by saponins (9.54±0.3 $mg.g^{-1}$), flavonoids (9.12±0.52 $mg.g^{-1}$) and then alkaloids (7.33±0.5 $mg.g^{-1}$). This result is supported by the study of Shah *et al* [22] and Salem *et al* [23]. In addition, this results relatively comparable to those reported in *Senecio glaucus* and *Urospermum picroides* as described by El-Amier *et al* [24,25].

AntioxidantActivity

The assessment of the antioxidant activity of the diverse plant extracts is presented in Fig. 1. By increasing the plant extract concentration there was a corresponding continuous increase in scavenging activity. In case of methanol, ethyl acetate, petroleum ether and hexane extractsthe increase was up to 4000 $\mu g.ml^{-1}$ where the scavenging activity was 53.52%, 50.48%, 37.53% and 36.39, respectively. The IC_{50} values of *S. marianum* extracts were presented in Fig.2. Methanol extract had the highest scavenging activity (1.99 $mg.ml^{-1}$). The radical scavenging activity of the other extracts and standard decreased in the following order: catechol, ethyl acetate, hexane and petroleum ether. These results suggest that methanol extract of *S. marianum* has an obvious effect on scavenging of DPPH radical. Similar results were reported by Salem *et al* [23] and El-Amier & Abdullah [26], while investigating the DPPH radical scavenging activity of *S. marianum* and *Senecio glaucus*, respectively.

Antimicrobial ActivityAssessment

Microbial disease and contamination have become one of the main problems of public health in the world, affecting all countries. It can be connected to the process of natural selection in bacterial development or the natural consequence of the adaptation of pathogen to exposure to antibiotics in the course of the indiscriminate use of antibiotics in humans and animals [27]. The antibacterial activity of *S. marianum* was assayed in vitro by agar well diffusion method against seven different bacterial strains. The part used for the study was shoot system and

four extracts (methanol, petroleum ether, hexane and ethyl acetate) were evaluated for antimicrobial activity as shown in Table 2 and Fig. 3. *S. marianum* extracts exhibited different inhibitory activities against the tested bacterial and fungal strains with different degrees as demonstrated by measuring the diameters of inhibition zones developed by the extracts. Table 1 showed that methanol and ethyl acetate extracts of

***S. marianum* showed the broad spectrumagainst**

The tested bacteria Fig. 4. Whereas, hexane extracts expressed an activity against *Shigella* spp and *P. vulgaris*, only. Petroleum ether extract showed the inhibitory activities against *E. coli*, *Klebsiella pneumoniae* and *Candidaalbicans*.

S. marianum produced inhibition zones less than that of the standard antibiotic ampicillin and clotrimazole against tested organisms. The pathogen E. coli was the most sensitive bacteria (25 mm), while A. fumigatus and A. niger were the most sensitive fungi (25 and 27 mm) in case of methanol extract. The present results agree with those of Izzo *et al* [28] and Dayanne *et al* [27] on same species. Ramdani *et al* [29] found similar results that the methanol extract of *Urospermum dalechampii* from Algeria was active against *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *B. subtilus* and *S. aureus*. El- Amier *et al* [24] also reported that *Sencio glaucus* (Asteraceae) extract showed an inhibition zone against *E. carotovora*, *S. biogensis* and *B. subtilis* but not against *S. aureus*, *E. coli* and *P. aeruginosa*. The main constituents of phytochemical analysis of *S. marianum* are silibinin, isosilibinin, silicristin, and silidianin (Sonnenbichler *et al* 1999). Silymarin has been found very active against most microorganisms [30,27].

AllelopathicActivity

The allelopathic potential of the *S. marianum*

Extract on the germination of C. murale 4daysafter treatment is presented in Fig. 5. The phytotoxicity of extracts was increased significantly with the increasing extract concentrations. At 25 g.l-1 and 20 g.l-1 the germination of C. murale was reached maximum inhibition (92.45% and 76.84, respectively). However, at the lowest concentration (5 g.l-1), the germination of C. murale was reduced by 14.26%. This agrees with the previous results of other investigators [31,32].

On the other hand, The allelopathic effect of the different concentration from extract of

S. marianum on *C. murale* radicle growth after10 DAT revealed that the higher concentration (25 $g.l^{-1}$) was strongly inhibited (97.23%) the *C. murale*, while the opposite response (26.17%) was observed at the lower concentration (5 $g.l^{-1}$) (Fig. 5). Similarity, the plant extract significantly reduced the plumule length of *C. murale* (Fig. 5). The highest concentrations (25 $g.l^{-1}$) of extract showed strong inhibition (87.29%) of plumule growth. The results of the prevailing studies agree with most of the previous results obtained by other researchers, which emphasized that extracts of many plant species inhibited germination of many other weed seeds [33,34,31].

It was noticeable that the degree of inhibition percentage of the plant extracts increased with the increase in its concentration. The present results showed the potent all elopathic effect of

S. marianum on the nuisance weed *C. murale*, which could be ascribed to the high content of phenolics, tannins and alkaloids. The reductioninthe germination of weeds could be attributed to the action of allelochemicals in the plant. Allelochemicals pose great effects on the membrane permeability, enzyme activities, cell division and ultrastructure, ion uptake and as a consequence germination, plant growth and development are modified [35,36].

CONCLUSION

In the present study, the *S. marianum* extracts exhibited different inhibitory activities against the tested bacterial and fungal strains with different degrees, methanol, and ethyl acetate extracts of

S. marianum showed the broad spectrumagainst the tested bacteria. The pathogen *E. coli* was the most sensitive bacteria (25 mm), while *A. fumigatus* and *A. niger* were the most sensitive fungi (25 and 27 mm) in case of methanol extract.

The phytotoxicity of *S. marianum* extracts was increased significantly with the increasing extract concentrations. At 25 $g.l^{-1}$ and 20 $g.l^{-1}$ the germination of *C. murale* was reached maximum inhibition (92.45% and 76.84, respectively). On the other hand, in case of *C. murale* radicle growth, the higher concentration (25 $g.l^{-1}$) was strongly inhibited (97.23%) the *C. murale*. Similarity, the plant extract significantly reduced the plumule length of *C. murale*. The highest concentrations (25 $g.l^{-1}$) of extract showed strong inhibition (87.29%) of plumule growth. Finally, this study showed that *S. marianum* extracts can be used as a natural antioxidant and antimicrobial agents in pharmaceutical as well as used in bio-control ofweeds.

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