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Investigation of phytochemical and antimicrobial properties of *Linum usitatissimum* in presence of $ZnO/Zn(OH)_2$ nanoparticles and extraction of euphol from *Euphorbia microsciadia*

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ABSTRACT

Linum usitatissimum and *Euphorbia microsciadia* are the two main plants species that are growing in vast diversity of Iran. These plant species have medicinal application in different regions of Iran. Nanoparticles are considered as greatest antibacterial agents for the prevention of bacterial growth on some medical materials. It seems these medicinal plants in presence of nanoparticles are effective tools to eliminate the severe bacterial infections. The hydroalcoholic extracts of these plants were obtained by maceration method. The extracts in presence of $ZnO/Zn(OH)_2$ nanoparticles were examined for the antimicrobial activity by broth macrodilution and agar disk diffusion. Additionally, the minimal inhibitory concentration and minimum bactericidal concentration were evaluated for the developed antibacterial materials. This retrospective study was done to survey the effect of plant extract with $ZnO/Zn(OH)_2$ nanoparticles suspension on bacteria and antioxidants content of the extracts were also determined. The result obtained demonstrated that the highest antioxidant activities associated with the shoot of *L. usitatissimum* (Total phenolic content) TPC1: 128.24 ± 1.127 mg gallic acid equivalents/g of dried extract, DPPH: 30.57 ± 0.4% inhibition and Total flavonoid 2: 95.04 ± 0.53 mg rutin equivalents/g of dried extract). Furthermore, euphol was isolated from acetone extract of *E. microsciadia* using thin layer chromatography and euphol's structure was characterized by high-resolution 1D and 2D NMR spectra. Combination of metal oxide nanoparticles with the aforementioned plants extracts can be effective in the eradication of the bacterial infections, and as a good alternative for antibiotics also.

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Keywords: Linum usitatissimum; *Euphorbia microsciadia*; Euphol; Antibacterial; Antifungal

1. Introduction

Throughout the history, plants have been used as a source of medicine and today scientists recognize their value as a source of new medicinal products or complimentary to currently developed medicinal products for augmentation purpose [1]. The plant-based medicine system plays an essential role in the health care as it is estimated that about 80% of population may be relaying on traditional medicines as a primary health care [2]. *Linum usitatissimum* (also known as common flax or linseed) is a member of the genus *Linum* in the family *Linaceae* [3]. With the advancement of techniques in the field of nanotechnology, it is plausible to develop new materials in the nanometer scale, including nanoparticles. These are usually defined as particulate materials with at least one dimension of less than 100 nanometers (nm), even the particles could be zero dimension in the case of quantum dots. Due to their distinctive features such as catalytic, optical, magnetic, and electrical properties [4,5], metal nanoparticles have been instrumental as these exhibit completely new or improved properties compared with larger particles of the bulk materials and these novel properties are derived due to the variation in specific characteristics such as size, distribution, and morphology of the particles. Nanoparticles present a higher surface area to volume ratio with decrease in the size of the particles. Specific surface area is relevant to catalytic activity and other related properties [6–8].

Zinc oxide is a chemical compound found naturally in the minerals [9]. Total phenolic and total flavonoid (TF) content of the extracts were also determined [10,11]. The antioxidant activity of these extracts were determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay and since plants are important source of antioxidant agents, researchers pay more attention to the plants' antioxidant properties [12]. Antioxidant properties keep cells away from the oxidative damage and diet rich with antioxidant may help to decrease the rate of some detrimental diseases like cancer, heart disease, and cerebral vascular accident (CVA) [13,14].

A variety of efficient, cost-effective, and environmentally friendly nanomaterials have been developed, each possessing unique functionalities in their potential application for the detoxification of industrial effluents, groundwater, surface water, and drinking water [15–17]. Also, used as adsorbents for removing ammonium ions in wastewater, nanomaterials should satisfy the following criterions: (1) The nanosorbents themselves should be nontoxic. (2) The sorbents present relatively high sorption capacities and selectivity to the low concentration of pollutants. (3) The adsorbed pollutant could be removed from the surface of the nanoadsorbent easily. (4) The sorbents could be infinitely recycled. As per earlier reported researches, a number of nanomaterials and nanoparticles have been synthesized and used as novel adsorbents for the removal of noxious pollutants from wastewater, and the previously obtained results indicate that these nanomaterials show high adsorption capacity [18–43].

In the present research, *Euphorbia microsciadia*, belonging to the family *Euphorbiaceae*, was utilized which is a native plant from Iran [44]. From the root of*E. microsciadia*, triterpene was isolated and the compound's structure was elucidated by high-resolution 1D and 2D NMR spectra and it was identified as Eupha-8, 24-dien-3β-ol (Euphol Fig. 4). The root extractions of *E. microsciadia* were performed in presence of dichloromethane, acetone, methanol, and methanol–water (80:20) separately and each of four extracts were screened for their antioxidant and antimicrobial properties.

2. Experimental

2.1. Plant material

L. usitatissimum and *E. microsciadia* Boiss (*Euphorbiaceae*) were collected from Dena mountain (near Sisakht, 2,980 m), Yasouj (Iran).

2.2. Part A

2.2.1. Synthesis of ZnO/Zn $(OH)_2$ nanoparticles

Zinc acetate dihydrate ($Zn(CH_3COO)_2{\cdot}2H_2O$) was used as zinc oxide source. It was purchased from Merck Company. Ammonia solution (25% w/w) was provided from Chem. Lab Company. These reagents were used further without any purification. The reaction solution for fabrication $ZnO/Zn(OH)_2$ nanoparticles was prepared as follows: 10 ml of 1 M zinc acetate solution was mixed with 10 ml of a fresh ammonia solution (25% w/w) at pH 10.5–11.0, distilled water was added to the mixed solution to make up to a total volume of 250 ml in a volumetric flask. In the next step, 100 ml of the prepared reaction

solution was transferred to a microwave oven. The reaction solution was kept in microwave oven for 30 min. Approximately after 5 min, the color of the reaction became milky white, it showed the slow formation of Zn(O, OH) nanoparticles. After 30 min, the reaction solution was removed from microwave oven and the obtained ZnO/$Zn(OH)_2$ nanoparticles were filtered and washed several times by distilled water. Finally, the prepared ZnO/$Zn(OH)_2$ nanoparticles were dried at room temperature.

2.2.2. Antimicrobial bioassay procedure

In vitro antibacterial and antifungal properties of root and shoot of *L. usitatissimum* extracts with ZnO/$Zn(OH)_2$ nanoparticles were tested against Gram-positive (*S. aureus:* ATCC 25293) and Gram-negative (*K. pneumonia*: ATCC 1827) bacterial and fungal (*A. oryzae* PTCC 5164). The biological activities of compounds were evaluated as inhibitory effect from the microbial growth by broth dilution and disk diffusion methods.

2.2.3. Antimicrobial screening using of broth dilution method

Broth dilution method was performed for the antibacterial tests using a serial dilution concentration of extracts and nanoparticles in DMSO solvent. The sterile test tubes including test sample solution, Muller Hinton broth as basal media, and bacterium were incubated at 37˚C for 24 h, then the minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) data were estimated [45]. The MIC of the compounds was assessed against a specified bacterium based on a broth dilution method. In this method, various concentrations of extracts with ZnO/$Zn(OH)_2$ nanoparticles were prepared in the sterile test tubes using the serial dilution method. Then, 0.65 mL of sterile Muller Hinton broth medium and 0.1 mL of bacterium were added to the test tubes and in final, the sets were incubated at 37˚C for 24 h. MIC is read as the lowest concentration of antibacterial agent that inhibits the visible growth of the test bacterium or that reduces the number of colonies of the test bacterium. MBC of extracts with ZnO/$Zn(OH)_2$ nanoparticles were also investigated [45]. In this method, we could observe bacterial growth on the surface of agar medium. MBC is read as the lowest concentration of antibacterial agent that kills all test bacterium (complete absence of bacterial growth).

2.2.4. Antimicrobial screening using of disk diffusion method

Antibacterial activity of the extracts with ZnO/$Zn(OH)_2$ nanoparticles was tested by using agar disk diffusion (ADD) method. A total volume of 100 μl of fresh bacterial culture was spread on nutrient agar and gently spread on the agar surface [45]. The bacterial concentration was 5×10^5 CFU/ml. For screening, 6-mm-diameter filter paper disk, impregnated with extract/nanoparticles solution (containing 0.4 ng and 80 pg of root's extract and ZnO/$Zn(OH)_2$ nanoparticles (1:1) for *K. pneumoni* and *S. aureus* plates. In continue 0.65 and 0.16 ng of shoot's extract and ZnO/$Zn(OH)_2$ nanoparticles (1:1) for *K. pneumoni* and *S. aureus* plates was used), was placed on the surface of inoculated agar plates. Incubation was carried out at 37˚C for 24 h. Clear zones of inhibition were measured in mm, including the diameter of the disk. Cephalexin and gentamicin (10 μg/disk) were used as control groups.

2.2.5. Antifungal screening by using of disk diffusion method

A. oryzae (PTCC 5164) were used for investigation of the antifungal activities of the extract with ZnO/$Zn(OH)_2$ nanoparticles by the disk diffusion method on the surface of Dextrose Agar inoculated with 105 (CFU/mL) of spore suspension of fungi. The Petri dishes of *A. oryzae* were incubated at 30˚C for 24–48 h. The disks impregnated in extract/nanoparticles solution (containing 12.5 μg of extracts and ZnO/$Zn(OH)_2$ nanoparticles (1:1 in 5% DMSO) were put at the different positions on the agar surface [46]. Finally, antifungal activities of compounds were evaluated as diameter of inhibition zone from the fungal strains growth. Antifungal activities of standard drug including amphotericin B have been presented.

2.2.6. Determination of total phenolic content

The total phenolic content (TPC) of the *L. usitatissimum* extracts was determined by using the Folin–Ciocalteu reagent (FCR) [10]. A total volume of 100 μl of the diluted ethanolic extracts containing 500 μg extract was mixed separately with (500 μl) FCR and diluted with distilled water and 0.4 ml of (7.5% w/v) sodium carbonate solution (Na_2CO_3). The solution was mixed and allowed to stand for 1 h at room temperature. Gallic acid solution (from 25 to300 μg/ml) was used as a standard reagent. Finally, the absorbance was measured at 765 nm using a UV–vis spectrophotometer. A calibration curve was prepared

by using standard solutions of gallic acid. The results were expressed as mg gallic acid equivalents (GAE)/g of dried extract.

2.2.7. Determination of TF

TF content of extracts was also determined [11]. A total of 1 mg of extracts was diluted with 1,000 μl of distilled water and 100 μl of 5% $NaNO_2$ solution were added. The mixture was kept at room temperature for 5 min and then, 200 μl of 10% $AlCl_3$ were added to it. This mixture was incubated at room temperature for a 6 min then 1 ml of 1 M NaOH was added to the mixture. The solution absorbance at 510 nm was measured with a UV–vis spectrophotometer. The concentration of the flavonoid compounds was calculated using the equation ($y = 0.01103x - 0.00507$: $R^2 = 0.9983$) that obtained from the rutin (50–500 μg/ml) calibration curve.

2.2.8. Scavenging effect on DPPH

Free radical-scavenging activity was estimated by DPPH assay using Von Gadow method with some modifications [12]. A total volume of 2.4 ml of DPPH radical solution (24 μg/ml) was prepared in 70% aqueous ethanol .The reaction mixture contained 100 μl of test extracts and 1 ml of methanolic solution of (24 μg/ml) of DPPH radical. The mixture was then shaken vigorously and incubated at 37˚C for 10 min. The absorbance was measured at 517 nm by using trolex solutions (100–1,000 μg/ml) as a standard. Lower absorbance of the reaction mixture indicates toward the higher free radical-scavenging activity which was calculated using the following equation: DPPH scavenging effects (%) = 100 × (Ac − As)/(Ac) where Ac is the absorbance of the control reaction and as is the absorbance of reaction mixture containing DPPH and extract at 517 nm.

2.3. Part B

2.3.1. General

^{1}H-NMR, ^{13}C-NMR, and ^{2}D-NMR spectra were recorded on a Bruker Avance AV 500 NMR instrument, using $CDCl_3$ as solvent. IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrophotometer and using $CDCl_3$ as solvent. Mass spectra were recorded by Agilent 5975C inert GC/MSD instrument.

2.3.2. Extraction and isolation of E. microsciadia

The roots of *E. microsciadia* after grinding were macerated with dichloromethane, acetone, methanol, and methanol–water (80:20) separately at room temperature for 2 d. After filtration, the extracts were concentrated under reduced pressure. The acetone extract was submitted to column chromatography to obtain pure compounds. The extract (4 g) was chromatographed over a silica gel column (200 g silica gel 70–230 mesh) using hexane with a gradient of dichloromethane up to 100% and then followed by ethyl acetate. The dichloromethane-rich fractions were then investigated by TLC. One of the fractions was pure (compound 4) and other dichloromethane-rich fractions were subjected to flash column chromatography (silica gel 230–400 mesh) by. Using hexane/acetone (0–30%) to yield 19 fractions: Frc1–Frc 19. Frc10 eluted in hexane/acetone 85:15, was purified further by TLC plate with chloroform. These fractions were treated for ^{1}H-NMR, ^{13}C-NMR, and ^{2}D-NMR spectra. Result of two compounds were reliable that reported here.

2.3.3. Determination of the total phenol content in the E. microsciadia extracts

The total phenol contents of the dichloromethane, acetone, methanol, and methanol–water (80:20) extracts were determined separately by the Folin–Ciocalteu method as described previously with some modifications [44,47,48]. Briefly, to a 40 μl of solution of each extract were added 3.16 ml of water and 200 μl of Folin–Ciocalteu reagents. The mixtures were shaken well and 600 μl of a 0.25% sodium carbonate was added to the each solution after 8.5 min incubation at room temperature. The reaction mixtures were kept in the dark and room temperature for 2 h and the absorbance of the solutions were measured at 765 nm against the blank. The concentrations of the total phenolics were measured against a series of gallic acid standard solutions and expressed as mg equivalent of gallic acid in 1 g of dry plant material (mg EG/g AM) [49].

2.3.4. Determination of the free radical-scavenging activity of the E. microsciadia extracts on DPPH Radicals

DPPH radical-scavenging activity was measured [48]. The extracts were diluted at the concentration of 0.63, 0.31, 0.16, 0.08, and 0.04 mg/ml. A total volume of 40 μl aliquots of the extract were mixed with 3,900 μl of the methanolic DPPH solution (40.78 g/L).

After 30 min of continuous shaking of the solutions in the darkness, the reduction of the DPPH free radical was measured by reading the absorbance at 517 nm. Inhibition ratio (percent) was calculated from the following equation: % inhibition = [(absorbance of control − absorbance of sample)/absorbance of control] × 100%. We used butylated hydroxyl toluene and quercetin as standards. The IC_{50} were calculated by linear regression equations of the DPPH inhibition percentage from different concentrations of the extracts [49–51].

2.3.5. Antibacterial activity bioassay

Antibacterial activities of the different extracts of *E. microsciadia* against various micro-organisms (Table 1) were determined by disk diffusion method. To examine that, three Gram-negative bacteria (*E. coli*: PTCC1330, *P. aeroginosa*: PTCC1074 and *S. typhi*: PTCC1609) and three Gram-positive bacteria (*S. aureus*: PTCC1112, *S. epidemidis*: PTCC1114, *B. subtilis*: PTCC1023) were chosen and tested in ADD bioassays. Bacteria were grown in high nutrient broth medium. Sterile disk was used for activity, saturated disk with the extract (0.04 ml) and known quantity of standard reference antibiotic separately was air-dried at room temperature. The media was inoculated and poured into sterile Petri plates. The disk with test extract was placed on the upper surface of sterilized plate that had been inoculated with the test organism and air-dried to remove the surface moisture. The thickness of medium was kept equal in all Petri plates and the standard disk (antibiotic) was used in each plate as control. The plates were inoculated 24 h at 37°C in incubator. After 24 h growth of bacteria was measured for its zone of inhibition. The results were obtained by measuring the zone diameter. The experiment was conducted in replicates of 3 and the mean value is presented. The results were compared with the control antibiotic [52,53].

3. Results and discussion

3.1. X-ray diffraction, SEM, and TEM analyses

The morphology of the prepared $ZnO/Zn(OH)_2$ nanoparticles was investigated by FE-SEM. It showed that the obtained $ZnO/Zn(OH)_2$ nanoparticles are mixture of the rod and the plate forms (Fig. 1). In continue morphology and size of the prepared $ZnO/Zn(OH)_2$ nanoparticles were also investigated by TEM technique. The TEM images of the $ZnO/Zn(OH)_2$–NPs with different magnifications were shown in Fig. 1(a)–(c). TEM images of the $ZnO/Zn(OH)_2$–NPs revealed the rod and plate forms shape of the developed nanoparticle combination. In TEM images, the rod nanoparticles were about 30 nm in diameter and an approximately 300–400 nm in length. On the other hand, nanoplates had a thickness about 20 nm and average diameter of 100 nm. Results were shown in Fig. 1(a), (b), and (c).

The X-ray diffraction (XRD) pattern revealed that the prepared $ZnO/Zn(OH)_2$ nanoparticles had polycrystalline shape and were combination of a hexagonal (zincite) structure of ZnO and a orthorhombic (wulfingite) crystal structure of $Zn(OH)_2$. Five strong peaks observed in diffractogram at about 20.22°, 20.95°, 27.26°, 27.85°, and 32.93°. They are in order related to (1 1 0), (2 0 0), (2 1 0), (2 0 1), and (2 1 1) planes of $Zn(OH)_2$ orthorhombic structure, respectively. In addition, three strong peaks observed at around 31.80°, 34.45°, and 36.3° are corresponded to (1 0 0), (0 0 2), and (1 0 1) planes of ZnO hexagonal structure, respectively. The strong XRD peaks were observed in Fig. 2, it reveals the well-crystallized structure of the prepared nanocomposite. On the basis of the full-width at half-maximum (FWHM) of (1 1 0) and (1 0 1) peaks and applying the Debeye–Scherrer equation, the average nanocrystallite size of ZnO and $Zn(OH)_2$ nanoparticles were estimated to be 30 and 20 nm, respectively. The estimated nanocrystallite sizes using the XRD data are in good agreement with TEM images of the particles.

3.2. Antimicrobial bioassays (in vitro)

MIC and MBC for root and shoot of *L. usitatissimum* extracts with $ZnO/Zn(OH)_2$ nanoparticles are presented in (Table 2). The compounds were tested against Gram-positive (*S. aureus:* ATCC 25293) and Gram-negative (*K. pneumonia*: ATCC 1827) bacteria. The antibacterial activities with agar diffusion method data of the compounds have been compiled in (Table 3). In *L. usitatissimum,* root extract/$ZnO/Zn(OH)_2$ exhibited higher antibacterial activities against all bacterial strains. Results showed that shoot extract has effective antibacterial properties in compare with antifungal properties but root extract has real antifungal effect. Antibacterial effects of metallic nanoparticles are stronger than other nanomaterial, which exhibit increasing chemical activity due to their large surface to volume ratios and crystallographic surface structure. Using the medicinal plant extracts with metal oxide nanoparticles can be effective to eliminate the bacterial infections, as an alternative to antibiotics.

The methanol extract of *E. microsciadia* had antibacterial effects on *E. coli*, *S. epidermis*, and *P. aeroginosa* with IZ of 11, 13, and 11 mm in the ADD tests, respectively.

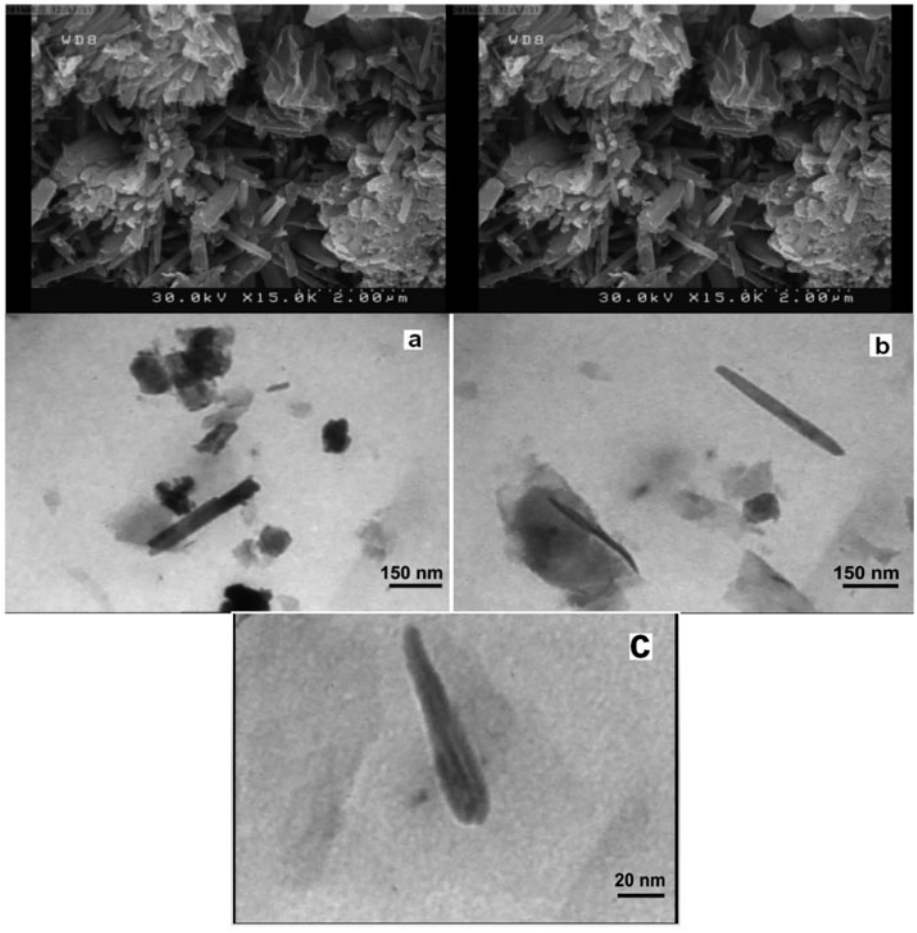

Fig. 1. FE-SEM image of the prepared $ZnO/Zn(OH)_2$ nanoparticles, TEM images of the prepared $ZnO/Zn(OH)_2$–NPs with different magnifications (a–c).

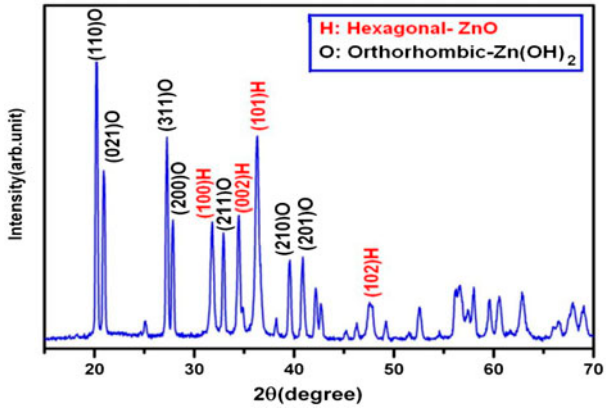

Fig. 2. XRD pattern of the prepared $ZnO/Zn(OH)_2$ nanoparticles.

While the DCM and acetone extracts of this plant did not have antibacterial properties. The methanol extract showed antimicrobial activity against 2 out of 6 micro-organisms including *E. coli* and *S. epidermidis* with IZ of 8 and 12.5 mm in the ADD tests, respectively (Table 1).

The antifungal activities of extracts with $ZnO/Zn(OH)_2$ nanoparticles as zone diameter of inhibition (mm) from the growth were tested against *A. oryzae* as fungal strains according to the defined method [54]. Antifungal activities of constructed disks showed considerable difference for compounds (Table 4). Root extract of *L. usitatissimum*/$ZnO/Zn(OH)_2$ was the more effective than shoot extract of *L. usitatissimum*/$ZnO/Zn(OH)_2$.

3.3. TPC and flavonoids

Phenols are the simplest bioactive phytochemicals having free radical-scavenging ability due to the presence of hydroxyl groups. The sites and the numbers of hydroxyl groups are related to their relative toxicity for micro-organisms, Recently, it is shown that

Table 1
Antibacterial activity bioassay

Cod	Bacteria	Methanol–water extract (80%) (mm)	Methanol extract (mm)	Antibiotic (mm)
PTCC1114	*Staph. epidermidis*	13	12.5	18
PTCC1330	*E. coli*	11	8	19
PTCC1074	*P. aeroginos*	11	_	23

Table 2
Review Sensitive of bacteria by Broth dilution method (MIC and MBC)

Compound	Organisms	MIC Extracts	MIC $ZnO/Zn(OH)_2$	MBC extracts	MBC $ZnO/Zn(OH)_2$
Root of *L. usitatissimum*	[b]*K. pneumoni*	0.37	0.3	1.49	1.2
	[a]*S. aureus*	93.1	74.5	0.37	0.3
Shoot of *L. usitatissimum*	*K. pneumoni*	0.728	0.582	1.45	1.16
	S. aureus	0.182	0.145	0.728	0.582

[a]Concentrations (pg/ml).
[b]Concentration (ng/ml).

Table 3
Antibacterial activity as diameter of zone of inhibition [a](mm) around the constructed disks

Compound (mg/disk)	*S. aureus* (Gram +)	*K. pneumonia* (Gram –)
Root of *L. usit.*/$ZnO/Zn(OH)_2$	12.4	11.4
Shoot of *L. usit.*/$ZnO/Zn(OH)_2$	11.3	10.6
Cephalexin	12	9
Gentamicin	10	9.44

[a]All data are the mean of three measurements.

Table 4
Antifungal activity as diameter of zone of inhibition [a](mm) around the constructed disks

Compound (mg/disk)	*A. oryzae*
Root of *L. usit.*/$ZnO/Zn(OH)_2$	10.6
Shoot of *L. usit.*/$ZnO/Zn(OH)_2$	10.46
Amphotericin B	11

[a]All data are the mean of three measurements.

increasing in hydroxylation of these compounds cause to increasing in their toxicity properties [55]. The phenolic contents of hydro alcoholic extracts of plants were tested using the diluted FCR. Phenolic compounds react with FCR only under basic conditions (adjusted by a sodium carbonate solution to pH 10). Dissociation of a phenolic proton leads to a phenolate anion, which is capable of reducing FCR. The reaction occurs through electron transfer mechanism. The blue compounds formed between phenolate and FCR are independent of the structure of phenolic compounds, therefore ruling out the possibility of coordination complexes formed between the metal centre and the phenolic compounds. It is believed that FCR contains hetero-poly-phosphor-tungstates-molybdates [56]. The highest contents of total phenolics were observed for shoot of *L. usitatissimum* (128.24 ± 1.127 mg GAE/g of dried extract) (Fig. 3(a), Table 5).

Flavonoids are polyphenolic compounds which play an important role in stabilizing the lipid oxidation and are also associated with antioxidative action [57]. Flavonoids found ubiquitously in plants and are the most common group of phytophenolics. Flavonoid content of the extracts in terms of (mg/g) rutin equivalents (RuE) was recorded (Table 5). The highest TF content of aqueous/ethanolic extracts was obtained from shoot extract of *L. usitatissimum* (128.24 ± 0.53 mg of RuE/g of dried extract). Data is shown in Fig. 3(b).

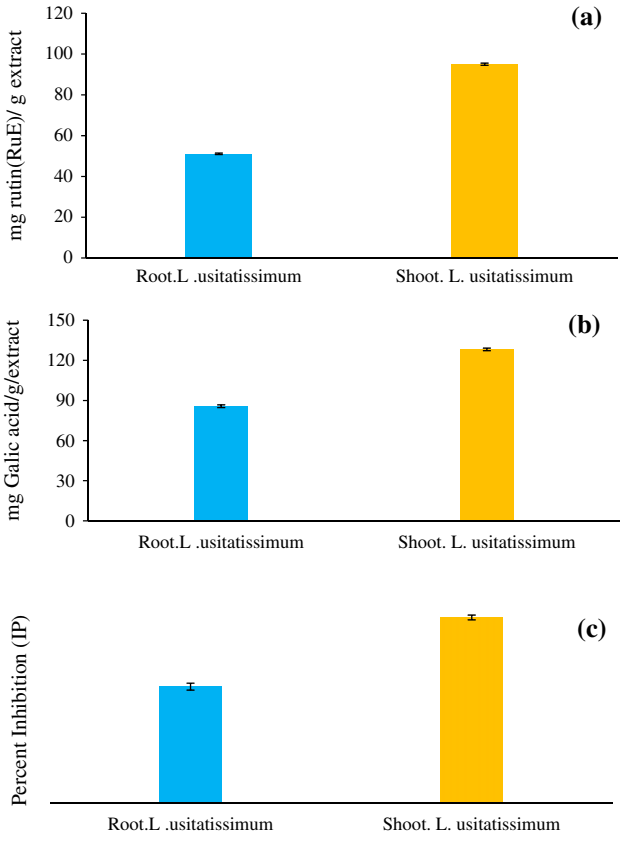

Fig. 3. (a) Comparison between phenolic content of the extracts, (b) Comparison between flavonoid; content of the extracts, and (c) Comparison of percent inhibition.

3.4. Antioxidant capacity

Recently, usage of antioxidants is proposed to protect people from oxidative stress damages. This study indicated that higher concentration of phenolic compounds in hydroalcoholic extracts improved antioxidant activity. Then these plants can be a use as a source of natural antioxidants to remove harmful effects of free radicals.

The *in vitro* antioxidant activities of test extracts were estimated using DPPH assay. DPPH radical-scavenging activity test measures the capacity of the extracts to scavenge the stable radical DPPH. If the extracts have this capacity, the initial blue/purple solution will change to a yellow color due to the formation of diphenyl picrylhydrazine.

Table 6
DPPH radical-scavenging potential

Sample name	IC50 (μg/ml)
Dichloromethane extract	811.8 ± 182.2
Methanol extract	159.4 ± 43.2
Methanol–water (80:20) extract	143.1 ± 31.2
Acetone extract	643.3 ± 249.2
BHT	7.2 ± 4.4
Quercetin	0.6 ± 0.2

The antioxidants when reacted with DPPH, a purple colored stable free radical which accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The amount of DPPH reduced was estimated by measuring the decrease in absorbance at 517 nm [58]. The highest DPPH radical-scavenging activity was obtained by aqueous/ethanolic extract of *L. usitatissimum* shoot (Bois leaves 30.57 ± 0.69%) (Fig. 3(c), Table 5).

Dichloromethane (DCM), methanol (MeOH), methanol–water (80:20), and acetone extracts of the *E. microsciadia* were subjected to the DPPH free radical-scavenging assays. Methanol–water (80:20) extract showed the highest radical-scavenging potential with IC_{50} = 1.78 ± 0.96 mg plant extracted to scavenge 1 mL of a 0.5 × 10–4 M DPPH solution (Table 6), and also (methanol–water (80:20) extract had the highest total phenol content 16.65 ± 1.00 mg GAE in 1 g of dry plant material (mg EG/g AM) (Table 7).

It was found that the shoot of *L. usitatissimum* had the highest antioxidant activities (TPC: 128.24 ± 1.127 mg GAE/g of dried extract, DPPH: 30.57 ± 0.4% inhibition and TF: 95.04 ± 0.53 mg cat chin equivalents/g of dried extract).

3.5. MS and NMR results

Compounds extract of *E. microsciadia* was purified using the acetone; the structure of the compound was elucidated using the mass spectra. The molecular

Table 5
Total phenolic, flavonoid content and antioxidant activity of hydroalcoholy extracts Results were mean ± standard deviation

Extracts	(GAE) Phenolic content (mg/g)	(RuE) Flavonoid content (mg/g)	(DPPH) inhibition (%)
Root of *L. usitatissimum*	85.73 ± 0.66	51.1 ± 0.37	19.17 ± 0.57
Shoot of *L. usitatissimum*	128.24 ± 1.127	95.04 ± 0.53	30.57 ± 0.4

Table 7
TPCs potential

Sample name	Total phenol content
Dichloromethane extract	5.14 ± 0.11
Methanol extract	14.75 ± 0.43
Methanol–water (80:20) extract	16.65 ± 1.00
Acetone extract	8.18 ± 0.33

Note: Total phenol (mg eq. gallic acid in 1 g plant).

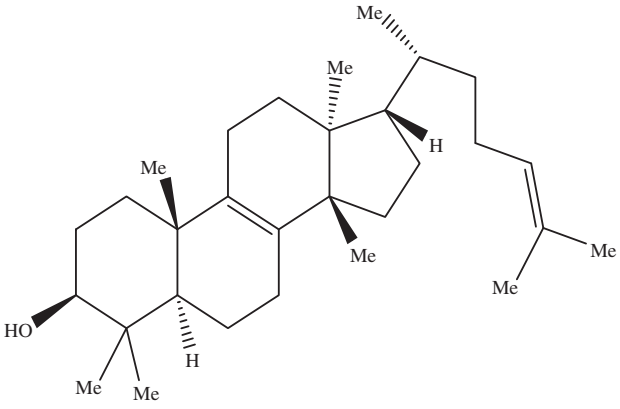

Fig. 4. Structure of Euphol.

formula is $C_{30}H_{50}O$ and also by high-resolution 1D and 2D NMR spectra such as DEPT, HMBC, and HSQC, we elucidated the correlation of carbons and hydrogen. Finally, the compound identified as Eupha-8, 24-dien-3β-ol (Fig. 4).

4. Conclusions

In the present study, antibacterial and antifungal properties of different extracts from *L. usitatissimum* and were screened against two types of Gram-positive and negative bacteria: *S.* aureus, *K. pneumonia*, and *A. oryzae*, using agar-well diffusion method and comparing their antibacterial activities with the antibiotics gentamicin, cephalexin, and amphotericin B. *L. usitatissimum* root extract demonstrated significant inhibitory effects against all tested bacterial and fungal. Moreover, *E. microsciadia* extract also showed antibacterial activities against *E. coli* and *S. epidermidis*, using the same concentration. *L. usitatissimum and E. microsciadia* are an abundant source of lignins, which have antioxidant properties and significantly reduce the effects of free radicals. The developed nanomaterial combination shows the TPC and free radical-scavenging activity. Phenolic acids and flavonoids are preventative in the decreasing rate of tumor growth and the decreased incidence of breast, prostrate, and colon cancers. *L. usitatissimum* is also a good source of flavonoids. They inhibit lipid peroxidation, platelet aggregation, and capillary permeability and fragility, thus leading to lowered incidence of cardiovascular diseases. Plants lignin is biologically important class of phenolic compounds. DPPH, a stable organic free radical, is very often applied for the evaluation of the antioxidant activity of compounds [1]. Free radicals are molecules possessing an unpaired electron emerging mainly in so-called oxidative stress, The main objectives of the present study were the qualification of antioxidant profile flax seeds using DPPH. This study showed that the process of degreasing has an influence on the antioxidant activity.

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