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Inhibitory Effect of Rhizomes Methanolic Extracts of *Rheum ribes* and Tio2 NPs on *Escherichia coli*

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Abstract

Rheum ribes is a traditional medicinal plant found in mountainous areas in northern Iraq, also present in Syria, Turkey and rarely in east Iran, was it used to treat various ailments. This study was aimed to investigate the effect of rhizomes methanolic extract of *Rheum ribes* rhizomes and Titanium dioxide nanoparticles (TiO2 NPs) against uropathogenic *E.coli*. The identification of chemical compounds was made by using GC-MS. Furthermore, the antibacterial effect of Rheum extract and TiO2 NPs was evaluated using well diffusion method. The results showed that Nine compounds were identified using GC-MS method and also Rheum extract was effective against bacterial isolates and the best effect in concentration 5000 µg/ml for all isolates (the inhibition zone 24 mm).TiO2 NPs showed good activity against bacterial isolates when it were soluted in D.W and in ethanol, so it can be concluded that they may act as antibacterial agents in the future.

Key words: E.coli; Rheum ribes; TiO2 NPs.

1. Introduction

Uropathogenic strains of *E. coli* (UPEC) form a subgroup of extra-intestinal pathogenic *E. coli* (ExPEC) strains was cause human urinary tract infections (UTI).

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Previous studies explained that there are many virulence factors associated with UPEC strains including adhesins, α-hemolysin and aerobactin production and cytotoxic necrotizing factor [1, 2].

Rheum ribes (Rhubarb), polygonaceae family, is a species of perennial and stout herbs that are found in the temperate and subtropical regions of the world, especially in Asian countries, *R. ribes* is a native plant which grows in north of Iraq, Turky, Iran and palastine. The local name is Rewas [3].

The plant was reported to have used in conventional medicine structure, the thick leaf-stalk of *R. ribes* is used as vegetable [4]. Leaf-stalk powder and roots are used to reduce disorders such as: Gastric illnesses, constipation, headache, kidney, bladder pain, uterine pain, and liver disorders, it also is used to increase appetite and bile secretion [5], and also used as antimicrobial activity against gram negative pathogens such as *Escherichia coli, Klebsiella pneumoniae, Proteus spp, Pseudomonas aeruginosa,* and *Neisseria gonorrhoeae* [6]. The antimicrobial activities of metal oxide nanoparticles and their discerning toxicity to biological systems propose their potential applications as therapeutics, diagnostics, surgical devices and nanomedicine-based antibacterial agents [7, 8, 9, 10]. Titanium dioxide nanoparticles (TiO_2) decompose organic compounds by the creation and constant liberate of hydroxyl radicals and superoxide ions when exposed to non-lethal ultraviolet (UV) light, which is highly proficient in inhibiting the growth of MRSA (11). This strong oxidizing power of TiO_2 NPs typically results in case of bacteria and other organic substances [12, 13, 14]. The growing application of drugs has resulted in the confrontation of pathogenic microorganisms to obtainable antimicrobial compounds. Hence, exploring and scheming alternative drugs from natural products is necessary to conflict microbial infections. Plant-based drugs, which possibly evolved as a chemical protection against predation or infection, is apparent to have less or no side effect compared with artificial antibiotics [15,16]. This study was aimed to investigate the antibacterial activity of Rhubarb extract and TiO2 NPs against uropathogenic *E.coli* and compare between each one of them.

2. Materials and Methods

Bacterial isolation and identification

Specimens of urine were collected in sterilized containers, in the laboratory within aseptic conditions; the collected specimens were streaked directly on MacConkey agar and EMB agar (Himedia/India) and incubated for 24h at 37ºC. Pink colonies were picked and recultured on another MacConkey and EMB agar. Further identification tests included the morphological characteristics and biochemical tests were carried out depending on [17] .Finally API E20 system was done.

Plant:

Rhizomes of *R. ribes* were collected from local markets of Karbalaa during October 2013. Authenticated by the Herbarium of Iraqi Ministry of Health according to a taxonomic method.

Preparation of plant extract:

Ten gram of *R.ribes* rhizomes was used for extraction. Methanol 95% (100 ml) extract were prepared by percolation. Extraction time was fixed for 24 h. The extract was filtered using Whatman No.1 filter paper. Sterilized by 0.22 µ micro filters [18].

Preparation of TiO2 NPs suspension

The preparation was done according to [19], one hundred milligrams of TiO2 NPs was added to 10 ml of sterile D.W and 100 mg in 10ml of ethanol, then shaken vigorously. The suspending solution was treated by ultrasound (40 kHz) for 30 min, autoclaved at 121 _C for 20 min and then cooled down to room temperature.

GC-MS analysis

GC-MS analyses were done by two methods:

***Method*:** according to [20] modified by [21]. Gas Chromatography – Mass Spectrometry GC-MS analysis used an Agilent 6890GC system coupled with an Agilent 5973N MSD operating at 70 eV, in lit temperature 200 °C; ion source temperature 200 °C; split injection (1 µl injection volume, split ratio, 50:1).; oven: 100 °C/min ; 275 °C at 10 °C/min for 20 min; transfer line temp.: 220 °C.Carrier gas helium; constant flow rate 1 ml/min; Capillary column (HP-5MS 30 m x 0.25 mm ID x 0.25 µm film, Agilent J & W, USA) was used; data acquisition by Agilent GC/MSD Chem-Station Version D.02.00.

Detection of antimicrobial activity of Rhubarb extract by well assay

The quantitative determination of Rhubarb extract activity against uropathogenic *E.coli* in bacterial culture was performed by using the wells assay that described by [22]**.** Different concentrations from the *R. ribes* crude extract were prepared by two fold dilution (500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90, 1.95 and 0.97) µg/ml. The *E.coli* isolates were put on Muller Hinton agar after comparsion with Macferland tube ($1x10^8$), wells were made in the plate by cork porer, different concentrations from the crude extract of plant was added in these wells. The clear zone was seen after overnight incubation**.**

Detection of antimicrobial activity of TiO2 NPs by well-diffusion method

The TiO2 NPs were used for studying the antimicrobial activity by well-diffusion method against uropathogenic *E.coli*. The untainted cultures of *E.coli* were subcultured onto sterile BHI broth and incubated at 37°C for 18 hrs.

After that the optical density of the above inoculated culture was recorded. Wells of 6-mm diameter were done on Muller–Hinton agar plates using cork porer. Each isolate was cultured uniformly onto the individual plates using sterile cotton swabs.

Using a micropipette, 100 µl from TiO2 nanoparticles NPs in different concentrations was added in each one of the wells. After incubation at 37°C for 24 hrs, the distinctive levels of zone of inhibition were measured.

3. Results and Discussion

Isolation and identification:

A total number of 30 urine samples were processed. Out of these samples, 16 isolates were confirmed as uropathogenic *E.coli* by morphological, cultural and biochemical tests. API E20 system was also done as confirmation test.

Determination of chemical compounds in Rheum ribs extract

GC-MS analyses

This method showed the presence nine compounds. The fragmentation patterns of the peaks were compared with that of the library of compounds. Nine compounds were identified using methods (1) (Table1), (Fig. 1). The major components present was 1'H-Cholesta-2,5-dieno[3,2-b]indole, 1'-(phenylmethyl)-(17.094%).

Table 1: Composition of *R.ribes* rhizomes methanolic extracts.

No.	Compound	Retention time (min)	Amount (%)	Chemical Formula	Molecular weight	Synonyms
1	Oxalic acid, cyclohexylnonyl ester	4.102	3.772%	$C_{17}H_{30}O_4$	298	no synonyms
2	Benzenepropanoic acid, α-(hydroxyimino)-	4.8	2.521%	$C_9H_9NO_3$	179	1.(2Z)-2-(Hydroxyimino)-3-phenylpropanoic acid #
3	4H-Pyran-4-one 2,3-dihydro-3,5-dihydroxy-6-methyl-	4.857	2.823%	$C_6H_8O_4$	144	1.3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one 2.2,3-dihydro-3,5-dihydroxy--6-methyl-4H-pyran-4-one 3.Pyranone 4.2,3-Dihydro-3,5-dihydroxy-6-methyl-4-pyrone 5.3-Hydroxy-2,3-dihydromaltol
4	1,3-Diazacyclooctane-2-thione	6.377	3.178%	$C_6H_{12}N_2S$	144	1.1,3-Diazocane-2-thione #

5	[1,1'-Biphenyl]-4,4'-diamine,	8.857	33.208%	$C_{38}H_{32}N_2$	516	no synonyms
6	Bis(diiodoarsino)methane	13.798	14.622%	$CH_2As_2I_4$	672	no synonyms.
7	Silane	15.024	14.622%	$C_{35}H_{74}O_2Si$	554	no synonyms.
8	1'H-Cholesta-2,5-dieno[3,2-b]indole,	17.222	12.557%	: $C_{40}H_{53}N$	547	1.7-Benzyl-1-(1,5-dimethylhexyl)-12a,14a-dimethyl-1,2,3,3a,3b,4,6,7,12,12a,12b,13,14,14a-tetradecahydrocyclopenta[5,6]naphtho[2,1-b]carbazole #
9	4,6-Bis(4-chloro-3-(trifluoromethyl)phenoxy)-2-pyrimidinol tbdms	20.471	7.237%	$C_{24}H_{22}Cl_2F_6N_2O_3Si$	598	1.Cl-1077 tbdms

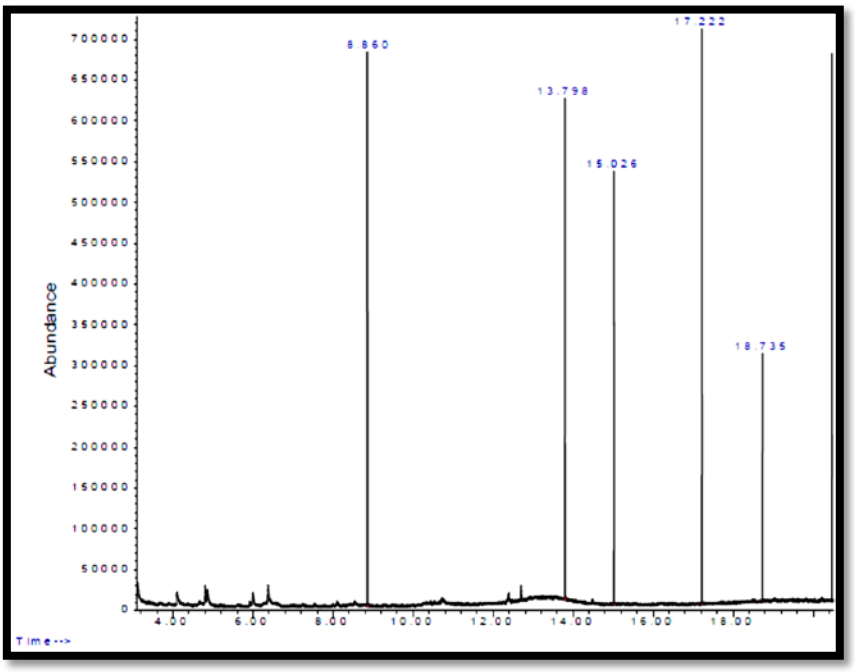

Figure 1: Chromatogram of *R.ribes* rhizomes methanolic extracts by GC-MS.

The method of GC – MS was given nine compounds, these compounds which have determined have pharmaceutical uses, some of them oxalic acid is a normal element in human blood and must be a available for the immune system to fight the diseases of cancer, viral, bacteria, and vascular conditions. When oxalic acid falls below an effective level the immune system can no longer protect the body from various diseases (23). Bis-(diiodarsino) methane this compound can be used in medicinal applications such as to induce apoptosis in human cancer cells and normalize abnormal cell growth associated with cervical dysplasia [24].

Detection of antimicrobial activity of Rhubarb methanolic extract by well assay

The results of the current study showed that Rhubarb extract gave antimicrobial effect against uropathogenic *E.coli* in all its concentrations, the higher effect was observed by higher concentration (5000 µg/ml) tha gave inhibition zone (24 mm), while lower effect by low concentration (19,53 and 9.76 µg/ml) tha gave inhibition zone with diameter (15 mm), as show in table (2) and figure (2).

Table 2: The antimicrobial effect of *R.ribes* methanolic extract by well diffusion method

Concentrations (µg/ml)	Diameters of inhibition zones (mm)
5000	**24**
2500	**21**
1250	**21**
625	**20**
312.5	**19**
156.25	**19**
78.125	**18**
39.06	**16**
19.53	**15**
9.76	**15**

Abdulla and his colleagues [25] in your study showed that the *R. ribes* ethanol and aqueous extracts display a broad spectrum of action against *E.coli, S. aureus, P.aeruginosa* and *P.mirabilis.*

The antibacterial properties of rhubarb are believed to have been caused by its embarrassment of enzymes in the mitochondrial electron +transport system [26].

Phenols are secondary metabolites largely concentrated in the plant kingdom with more than 8,000 phenolic structures presently known, ranging from simple molecules, such as phenolic acids, to highly polymerized substances, such as tannins. Positive correlations between total phenolics and antioxidant capability have been reported [27].

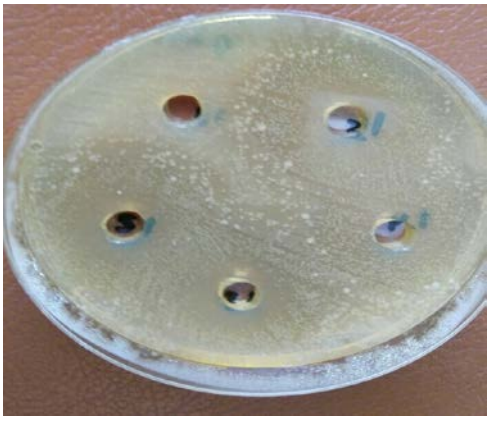

Figure 2: Antibacterial effect of Rheum methanolic extract against *E.coli*.

From the results above it can noticed that Rheum extract can be developed as new antibacterial agents in order to avoid bacterial resistance and achieve the desired clinical effect.

Determination of antimicrobial activity of TiO2 NPs by well-diffusion method

The antimicrobial activity of TiO2 NPs was investigated against uropathogenic *E.coli* using well diffusion method. The width of inhibition zones (in millimeters) around each well with TiO2 NPs solution is represented in table (3). It was found that at (250,125, 62.5, 31.25, 15.62) µg/ml concentrations from TiO2 NPs soluted in D.W were capable to inhibit bacterial development but at (7.8, 3.9, 1.95, 0.97, 0.480**)** µg/ml there was no effect, while the last 4 concentration gave antibacterial effect as show in table (3). On the other hand it was found when TiO2 NPs diluted in ethanol gave the inhibition zones approximately the same as that found when these nanoparticles diluted in D.W. The greatest zone of inhibition was 40 mm at 0.030 µg/ml and least zone of inhibition was 18 mm at 15.62 µg/ml were observed when NPs diluted in D.W., whereas the highest zone of inhibition was 32 mm at 500 µg/ml in NPs diluted in ethanol and less one was 16 mm at 31.25 µg/ml.(see figure:3)

Figure 3: Antimicrobial effect of Tio2 NPs against *E.coli*

Table 3: The diameters of inhibition zones for Tio2 NPs against uropathogenic *E.coli*

Concentrations(µg/ml)	NPs in D.W (mm)	NPs in ethanol(mm)
500	**No effect**	**32**
250	**32**	**30**
125	**29**	**28**
62.5	**25**	**26**
31.25	**21**	**16**
15.62	**18**	**_**
7.8	**_**	**_**
3.9	**_**	**_**
1.95	**_**	**_**
0.97	**_**	**_**
0.48	**_**	**_**
0.24	**38**	**30**
0.122	**38**	**24**
0.061	**32**	**20**
0.030	**40**	**18**

The results of present study indicated that TiO2 NPs soluted in D.W gave highly effect against uropathogenic *E.coli* in compare with TiO2 soluted in ethanol. The larger inhibition zones that achieved by TiO2 in D.W. were (32, 38, 40 mms) in these concentrations (250, 0.061, 0.24, 0.122 and 0.030) µg/ml, see table (3).

Tio2 NPs soluted in ethanol also gave inhibition zones ranged from (16-32), the largest inhibitin zone was achieved by concentration 500 µg/ml.

Gelover and his colleagues [28] reported that TiO2 NPs are the commonly studied for their photocatalytic antimicrobial action among several NPs. Roy and his colleagues [29] in their research recommended that TiO2 NPs abortive to exhibit antibacterial activity, but upon mixture with antibiotics they were capable to slow down the growth of microorganisms.but here TiO2 NPs without any type of mixing was able to inhibit uropathogenic *E.coli.* The TiO2 photocatalysts have been investigated widely for the murder or growth slow down of bacteria, with the benefits of physicochemical stability, nontoxicity, bio-compatibility, and low-cost [30, 31].

Being strong oxidant, the reactive oxygen species generated by the TiO2 photocatalytic reactions cause a variety of reparation to microorganisms ensuring their fast inactivation. Matsunaga and his colleagues [32] reported for the first time the microbiocidal effect of the platinised TiO2. Since then investigation work on antibacterial property of TiO2 has been intensively conducted against a broad spectrum of pathogenic microorganisms including bacteria, viruses, fungi and algae [33, 34].

4. Conclusions

The results of this research showed that methanolic extract of *R.ribs* had antibacterial activity against uropathogenic *E.coli* in different concentrations of it. This paper also reported that TiO2 NPs were effective against pathogenic bacteria in planktonic state at different concentrations.

5. Recommendations

R. ribs extract can be used as antibacterial agent against other pathogenic species, however different extract of another species of *Rheum* should be tested against different type of bacteria.

In other studies can be used different types of nanoparticles against different bacterial species and compare the effect of it against planktonic bacteria and biofilm.

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