ORIGINAL ARTICLE

A comparative preliminary investigation of the effect of hot and cold extract of Rubia tinctorum L and Study the antibacterial effect of Rubia tinctorum nanoparticle extract against some pathogenic bacteria

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Abstract

*Rubia tinctorumL.*kown as fuwaa in Iraq is one of the important plants used in traditional medicine such as treating urinary tract stones and infection s. This study was conducted for comparative preliminary investigation of the effect of hot and cold extracts of R. *tictorum* roots. The hot extraction was carried out by soxhlet apparatus while cold extraction was done by maceration. In both methods ethanol 90 was used. Study results show that hot extracts contain alkaloids while cold extract contain alkaloids, tannins and phenolic acid respectively. The main aim of this study was to investigate the preliminary phytochemical and evaluate the antibacterial potential of silver nanoparticle synthesized from cold and hot plant extracts of *Rubia tinctorum* . The use of nanoparticle in medicine is an attractive proposition. In the present study,silver nanoparticle were evaluated for their antibacterial activity. This study was designed to assess the antibacterial effect of *Rubia tinctorum* extract nanoparticle hot methanolic and cold methanolic in *vitro* with the use of nanoparticle against some pathogenic bacteria like gram positive bacteria like *Staphylococcus aureus* and gram negative bacteria like *Pseudomonas aeruginosa*, E.*coli*,*Proteus vulgaris*. Therefore, we establish acombination of medicinal values of and nanotechnology possibly with the field of medicine for the development of antibacterial agent these four bacteria. The hot and cold extract showed various degree of inhibitory activity against four species of bacteria.The synthesized silver nanoparticle were characterized by UV visible spectroscopy. The antibacterial activity of silver nanoparticle activity of silver nanoparticle prepared from against these bacteria.The silver nanoparticle show high antibacterial activity when assayed by agar well diffusion method. This green synthesized nanoparticle could be used in the medical field against human disease due to their high efficiency as antibacterial agent. Inconclusion,silver nanoparticle and R.*tinctorum* herb extract act as potent antibacterial agents and may prove to be better antibacterial against wide range of microbes.

Keywords: Antibacterial activity, R.*tinctorum*, hot extract, cold extract , Silver nanoparticle

1. INTRODUCTION

Rubia tinctorum L. or madder plant also known in Iraq as fuwaa plant [1] is a herbaceous perennial plant which grows widwly in different cold regions such as north of Iraq [2]. Rubia genus of flowering plants belong to Rubiaceae family [3]. Roots and rhizomes of Rubia tinctorum L. considered as the medicinal parts used in the treatment of diseases which collected in spring or autumn seasons in the third or fourth year of cultivation [4]. Madder plant has been considered as economical uses [5]. R. tinctorum roots considered as a natural dye (a kind of pigment) used as food additive used for wool or fibers pigmentation [6, 7].

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Fuwaa roots have different active compounds such [8] as tannins, as anthraquinones especially lucidine, primeveroside, ruberthric acid, pseudopurpurin, mungistin, alizarin,1,2-dihydroxoxoyanthraqunone and purpurin [9]. The red color of R.tictorum roots due to alizarin components: roots have high content of tannins which is used for the treatment different digestive system proplems [10]. Rubia also has other compounds such as anthraqunone, naphtoqunone, cyclic hexapeptide, trepenoids and different types of compounds [11]. The water extract of R.tinctorum roots was used for induced jaw edema in rat [12] ;also roots are used for hematemesis, epistaxis, flooding, spitting, traumatic bleeding, amenorrhea caused by obstruction , joint impediment pain, swelling and pain caused by injuries from falls [13]. Iraqi people used fuwa roots water extract for urinary tract diseases; therefore, this study was conducted by investigate the effect of hot and cold extraction of R. tinctorum active compounds.

Plant are rich sources of various medicinally important substances and they explore the huge diversity in therapeutic. Medicinal plant is a source of many potent and powerful drugs and used all over the world for the treatment of chronic disease [14].

The root of Rubia. tinctorum (madder) are the source of natural dye. The dye components are anthraquinones which probably contribute to the resistance of plant against fungi in the soil [15]. These herbs that have been used for treating inflammatory diseases. Bisswas et al [17] shows the use of this extract in the treatment of wounds and injuries in the traditional medicine. R.tinctorum used in the treatment of urinary bladder infection [18]. Numerous-phytotherapeutic literature data describe usage of these plant in the treatment of bladder infection as traditional folk remedies. Tea is prepared out of R. tinctorum and it is suggested form of remedy according to the folk medicine knowledge. [19]. R.tinctorum is a part of the Rubiaceae family and it is kown as rich with antraquinone which is used as anti-inflammatory antimicrobial , antibacterial , and antidiuretic drug. Antraqinone for R. tinctorum which is denoted as amain antibacterial compound, it is nessesery to stress that antraqinone is porly soluble in water as in ethanol on temperature lower the boiling water [20]. Previous study demonstrated antibacterial effect of R.tinctorum on pathogenic bacteria but not on E. coli. R.tinctorum contain spolyphenol lies on the potential to oxidize and on hydrolyze the bacterial cell wall and plasma membrane [21]. Nanotechnology and their derived products are unique not only in their treatment but also in particle size, physical, chemical and biochemical properties [22]. Nanotechnology provides a good platform to modify and develop the important property of metal in the form of nanoparticles application in biomarkers, cell labeling, antibacterial agents, and nanodrug for the treatment of disease. [23 ,24]. Silver nanoparticles have important biological pro samples were kept for perties, they are effective bactericidal agents against broad spectrum of bacteria [25]. Silver nanoparticle of 5-20 nm diameter can inhibit HIV-1 virus replication [26, 27]. Nanotechnology deals with the synthesis of nanoparticle with controlled size, shape, and dispersity of materials at the nanometer scale length. [28, 29, 30]. The aim of the present study was to investigate the preliminary phytochemical investigation and the antibacterial potential of silver nanoparticles with hot and cold extract of R. tinctorum.

2. MATERIALS AND METHODS

1-Sample preparation

R.tinctorum roots were obtained from Sulaimania region in the north of Iraq;the sample was identified and authenticated by Iraqi National hebarilism. The sample were kept for drying at the lab in pharmacnogosy and medicinal plant department college of pharmacy University of al Mustansiriya and then grinded for extraction procedure.

2- Extraction methods

A/hot extraction

Rubia roots were thoroughly washed, dried in the shades and then grinded by a mechanical grinder. 25 g of rubia roots powder was packed in the thimble of Soxhlet apparatus and extracted with 250 ml 90% ethanol for 12 hours, then the extract was concentrated evaporator to get a dry residue. vacuum using tottery evaporator to get a dry residue.

B/cold Extraction

25 g of rubia roots powder was macerated with 90 % ethanol for one week, then filtered and concentrated under vacuum using rotary evaporator to get a dry residue.

Preliminary phytochemical investigation
Preliminary investigation for the active chemical compounds was done by using the following chemical tests.

Alkaloids
2ml of extract was taken separately with 5ml of 1.5%v/v aqueous hydrochloride acid and filtered. The resulting acidic solution tested with Mayers Wanger and Dragendroffs precipitate was observed on addition reagent, indicate the presence of alkaloids. Development of orange precipitation on addition of drangendroffs reagent is the positive test for alkaloids.

Flavonoids
2ml of aqueous solution with few drops of KOH give yellow color and then added few drops of diluted acid give colorless.

Tannins
0.5ml of alcoholic and water extract were diluted with 1ml of water and 2-3 drops of dilute ferric coride solution was added. Development of aback/ green colour indicate the presence of tannin.

Phenols
The dried residue of each extract was dissolved in methanol. Methanolic extract was tested for the presence of phenolic. A few drops of acidified ferric chloride 5% solution were added to the extract. The presence of blue, green or brown coloration indicate the presence of phenolic compound in the sample.

Cardiac glycosides
1ml of glacial acidic acid and was added to 2ml of methanol extract in test tube. In this mixture few ml of ferric chloride followed by 2 drops of concentrated H2so4 were added. Green blue color indicates the presence of cardiac glycoside (killer- Killian test).

Collection of bacterial strain
The bacteria were collected from the department of microbiology from the hospital of Yarmuk between 1 to 15 May 2018.

In vitro testing of extract
The cup plate agar diffusion method activity of the extract. One ml of the isolated bacterial stock suspension (10 os 8 to 10 os 9 CFU were thoroughly mixed with 100 ml of molten sterile Mueller Hinton agar, 20 ml of liquots of the inoculated Muller Hinton agar were distributed into sterile petri dishes. The agar was left to set and all these plates 4 cups 10 mm in diameter were cut using a sterile cork borer no 4 and agar disks were removed. The cups were filled with o.1 sample of two extract use of automatic microliter pipette, and allowed to diffuse at room temperature for two hours. The plates then incubated in the upright position at 37 c for 24 hours. The plates were observed for the presence of inhibition of bacterial growth that was indicated by clear zone around the wells. The size of the zone's inhibition was measured and the antibacterial activity was expressed in terms of average diameter of the zone of inhibition in millimeters. The results were compared with the standard antibiotics.

Synthesis of silver nanoparticle
10 ml of ahot and cold methanol was added to 100 ml of 0.01 m AgNo3 solution in a conical flask at RT. The color of the solution cha. The UV was changing with in 15 min from yellow to brownish black (3 %) dark yellow to dark brown color 5 %, indicating formation of nanoparticle exhibit color change solution due to excitation of surface plasma vibration in silver nanoparticle the UV vis spectroscopy of the synthesized UV S spectral analysis was done by using UV vis spectrophotometer. Afinal solution of was centrifuged as synthesized nanoparticle as 8000 RPM for 25 minutes. The collected pellets were stored at -4c and supernatant was discarded.
Screening of green synthesized nanoparticles by Disk diffusion method

Culture of E. coli, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus was obtained from the all Yarmuk Hospital . Standard size whatman no 1 filter paper disk 6mm in diameter antibacterial activity. Nutrient agar medium for disk diffusion test was prepared after sterilization petri dish and allowed to solidify. The culture separately a particle by disk diffusion method filter paper disk were soaked in diluted (100 %).

Ecofriendly synthesized nanoparticle. Synthesized nanoparticle disk of 100 mg /ml per dish was placed on the an agar plate containing bacterial suspension . Similarity, solution of standard antibiotics streptomicin sulphate of 100 mg / ml .

3. RESULTS

The results of phytochemical screening for R.tinctorum are shown in Table 1 which reveals the presence of Alkaloides, phenol, cardiac glycosides, Flavonoides ,terpens, Tannns<Ratengens, Coumarines

and essential while in the cold extraction alkaloids, tannins, and phenolic acids were present and cardiac glycosides were absent in both hot and cold extraction methods as shown in table (1). oil. Study results revealed in the hot extraction method the presence of alkaloids only;

In the present study we evaluated the possible therapeutic effect of silver nanoparticle and R.tinctorum on pathogenic bacteria.

One natural dye was screened for their antibacterial activity against four test bacteria and the first screening showed that R. tictorum hot and cold methanolic extract showed inhibition on gram positive bacteria like S.aureus and less on gram negative bacteria (Table 2), but this effect increase when combination with silver nanoparticle (Table 3).Table 4 shows the effect of silver nanoparticle alone and with hot and cold R.*tinctorum*.

4. DISCUSSION

As mention in results we found that alkaloids were present in both hot and cold extraction method agree with [3] Nanoparticle synthesis is an emerging area of research in the scientific world. Green chemistry procedure, probably involving organisms would benefit the development of clean, nontoxic, and environmentally acceptable synthesis nanoparticle [31,32].

In the present study, the extract of the examined plants was an easy eco-friendly and cost-effective process to prepare NPs by reduction of silver nitrate with R. tinctorum obtained with that solved may natural hydrophilic product. [33].

The present study agreement with Branislav Radojica et al [34] who report Aqueous extracts of R.tinctorum and S. teetorum have higher antibacterial potential than their ethanol extract, and agreement with Yadav et al [35] . The folk medicine of almost around the world rely chiefly and alarge numbers of drugs are derived from plants. The therapeutic uses of plant are safe and economical and effective as their use of availability. Previous studies on antibacterial potential of different medicinal plant (ag) present in the solution of silver complex in the plant extract demonstrated that the change in colour was due to the formation of silver nanoparticle in the solution which are correlated with the UV-V visspectra [36] also support our results and efficacy of silver NPs [37, 38]. The hot and cold extract using plant extract and bacteria. The reduction of silver ions interested in synthesizing nanoparticle of different shape and size by employing bio-based synthesis of nanometals. The key to their significant bactericidal activity is the ability of silver nanoparticles to release silver ions more specifically. The high specific surface to volume ratio of silver nanoparticles increase their contact with micro organisims, likely to be promoting the dissolution of silver ions improving biocidal effectiveness [39]. The present study agreement with Monores et a 2005 [40] who report silver nano particless having size in the range of 10-100 nm showed strong bactericidal potential against both Gram positive and Gram-negative bacteria. Further investigation regarding the mode of action and their related pharmacological studies such as in vivo investigation, drug formulation and clinical trials highly recommended. Application of such eco-friendly nanoparticles method potentially exiting for large scale synthesis of silver nanoparticles from R. tinctorum

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Table [1] Chemical tests results of the hot and cold extraction methods

Active compounds Hot	Hot textract	Cold extract
Alkaloids	+	+
Flavonoides	-	-
Tannins	-	+
Phenols	--	+
Cardiac glycosides	-	-
+presence	-absence	

Table [2]. The antibacterial activity of cold and reference antibiotic against the isolated bacteria

Tested bacteria	100	50	25	12.5	6.25	Gentamicin
E.coli	20-+0.21	18	17	15	-	30
P.vulgaris	10+_0.1	-	-	-	-	25
P.aeruginosa	10+_0.1	-	-	-	-	25
S.aureus	16+_0.1	15	14	12	8	23

Table [3]. The antibacterial activity of hot and reference antibiotic against the isolated bacteria concentration mg / ml

Tested bacteria	100	50	25	12.5	6.25	Gentamicin mg/ml
E.coli	19+_0.2	18.02	16+_0.1	15+_0.1	10+0.1	30
P.vulgaris	10+_0.1	-	-	-	-	25
P.aeruginosa	12+-0.1	-	-	-	-	25
S.aureus	18+_0.1	16.01	15+_0.1	13+_0.1	12.+_0.1	24

Table [4]. The effect of silver nanoparticle alone and the hot and cold R. tinctorum

Tested bacteria	Inhibition zone (mm)		
	HotR.tictorum AgNPs	Cold.T.tinctorum AgNPs	Silver nanoparticle alone
E.coli	12	10	5
P.vulgaris	12	11	4
P.aurgenosa	13	11	7
S.aureus	12	11	6