Research Article

Evaluation of anthelmintic and antimicrobial activity of Ursolic acid obtained from Tulsi (*Ocimum sanctum*)

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Abstract

Objective: This study was designed to extract ursolic acid (UA) from *Ocimum sanctum* and to evaluate the anthelmintic activity and antimicrobial effects on the *E coli*. Ursolic acid, an important bioactive compound, was isolated from ethanolic extract of *Ociumum tenuiflorum* plant also known as *Ocimum sanctum*. **Materials and methods:** Ursolic acid was isolated from the ethanolic extract of plant *Ocimum sanctum.* It was evaluated for anthelmintic activity and antibacterial action on *E. coli* with disc diffusion and well diffusion method. Compare the anthelmintic activity and antibacterial action of ursolic acid with standard drug albendazole and tetracycline respectively. **Results:** The evident obtained from the experimental data, ursolic acid showed significant anthelmintic activity against earthworm *Pheretima postuma*. Screening of compound was performed for complete death and paralysis of worms. The results reveal a dose dependent increase in activity of the compound at 15, 20 and 25 mg/ml concentration. Results were comparable along the standard drug albendazole, in the same concentration. The results of antibacterial screening assay of ursolic acid by disk diffusion method and well diffusion method. The *in vitro* anti-microbial studies reveal that the ursolic acid was potentially active against microbial species and exhibit greater biocidal effect as compared to ligand fragments. **Conclusion:** The present study shows the potent anthelmintic as well as antibacterial activity of ursolic acid that was good in their action against the worms. The results indicated that ursolic acid have the potential to paralyze and kill the parasitic worms.

Keywords: *Ocimum sanctum*, ursolic acid, anthelmintic activity, antibacterial activity

Introduction

The mechanisms by which all natural compounds protect themselves against different stressors are complex and are in varied range. Individual phytoconstituents have been used as modulators of many metabolic and regulatory reactions. *Ociumum tenuiflorum* also known to the world as *Ocimum sanctum*, Holy basil or Tulsi is an aromatic plant with number of properties. It is native throughout the Eastern world and tropics and is widely cultivated throughout the world. The variety of *Ocimum tenuiflorum* used in Thai dishes is known as Thai Holy Basil (Staples and Kristiansen, 1999). Ocimum is a genus of which have around 35 species of aromatic annual and perennial shrubs and herbs. Some species includes *Ocimum basilicum* or Thai basil; *O. gratissimum* or African Basil, *O. campechianum* or Amazonian basil; *O. tenuiflorum* or *O. sanctum* or Tulsi or Holy Basil; *O. citriodorum* or Lemon Basil, *O. sanctum* grow up to 60 cm high with purple or red sub quadrangular branches. Leaves are simple, serrate and hairy. Flowers are purple in color. Fruits are smooth in touch and not mucilaginous when get wet. It is produced by means of seeds. Seeds are planted directly in the ground. Young plants are transplanted to the field when they attain 8-10 cm height (Vijayalakshmi et al., 1997). Tulsi shows active action to reduce disease of the head and neck, swelling, pain, headache and skin disorders. Tulsi leaves have very potent for lung intestinal and cardiovascular diseases. Tulsi leaves are also very effective in blood sugar, blood cholesterol and reducing stress.

Ursolic acid, is an ursane-type pentacyclic triterpene, it is a

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constituent of certain medicinal herbal plants and is also found in fruits of different plants (Liobikas et al., 2011). This triterpenoid is the major secondary metabolite can be obtained by the isolation of alcoholic extract of the aerial parts of Ocimum tenuifloruma shrub, popularly known as "holy basil". It is well known to have a wide range of properties to work against number of ailments including anti-inflammatory (Ku and Lin, 2013), antineoplastic (Shanmungam et al., 2013), reduces blood sugar level (Alqahtani et al., 2013), antimalarial against (Vanet al., 2006), antioxidant (Liobikas et al., 2011; Ali et al., 2007; D'Abrosca et al., 2005), antimicrobial (Acebey-Castellon et al., 2011), and prevents abdominal obesity (Rao et al., 2011). Antibacterial actions of pentacyclictriterpenes and their derivatives have been extensively studied (Mallavadhani et al., 2004; Cunha et al., 2010; Silva et al., 2012), and the activity of these compounds also have in their potential to enhance bacterial susceptibility to other substances, including antibiotics (Kurek et al., 2012). Antibiotic resistance to various diseases is a serious problem in the area of patient health. There is a tough challenge for the researcher to search new therapeutic agents that can help patients infected by bacterial agents. The natural products are showing good results as a better alternative.The formation of oxygen and free radicals inside the body, sometimes involves in the development of many diseases such as rheumatoid arthritis, inflammation, Alzheimer's diseases, Parkinson's andcancer (Bano et al., 2012). The usual antioxidants possesminimum toxic side effect and show protection from oxidative degradation by lowering free radicals in pharmaceutical, food and cosmetic. The purpose of the recent study was to determine the anthelmintic and antibacterial activity of ursolic acid from *Ocimum sanctum*.

Worm infestation (helminthiasis) is considered as a major in African countries where health problem is a big issue. The worms enter in the form oflarvae or egg inthe human body through several ways like by mosquitoes (filarial worms), direct contact, infected food, and water and soil. Helminthiasis results in several related diseases and is very harmful to humans and animals. Few anthelmintic drugs are available in the market to kill and remove all parasitic worms from the infected host body. Worm (Helminthiasis) infection is a general infection in person, disturbing a large crowd of the globe. Helminthiasis is the type of gastric disease, which cause affects the productivity of various pets as well as small ruminant live stocks. The worms is now a days great threat to health and can produces variety of disorders like malnutrition, pneumonia, anemia etc. Parasitic infections are prevalence but the research is limited in anthelmintic drug.

The gastro-intestinal helminthes happen to resistant to presently existing drugs. Developing novel anthelmintic compounds for chemotherapy which can be used toavoid the problems of drug resistance may be a crucial weapon. In the earlier period, many decades since penicillin was revealed and initiated as a dominant antibacterial agent, antibiotics arebecoming critical in the fight in opposition to communicable diseases occurred because of bacteria and other microorganisms. Nevertheless, prevalent use of antibiotic has encouraged the appearance of pathogens resistant to antibiotics, as well as multidrug resistant strains. At current, the occurrence of more and more species of pathogenic bacteria dead set against to conventional antibiotics has effected in either high operating expense or disappointment in the cure of infectious diseases. An alarming enhancement in resistance of community acquired infectious bacteria that have also been recognized, particularly in Pneumococci and Staphylococci, which are widespread reason of disease and death. In addition, a mainsource of mortality for patients rapidly accompanied with AIDS disease, and as visibly consequence invasive infections represent the risk of opportunistic fungal infections is increased. With the coming out of innovative microbial strains which are resistant to numerous antibiotics conventionally available, so interest in the discovery of new antibacterial agents are being grown.

Materials and methods

The plant leaves of Tulsi (*Ocimum sanctum*) were obtained from Botanical garden of Rani Durgavati University, Jabalpur MP, India, during the month of March-April 2015. Albendazole and Tetracycline were obtained from Pharmaceutical Chemistry Laboratory and test sample ursolic acid was synthesized in Pharmaceutical Chemistry Laboratory, Department of Chemistry and Pharmacy, Rani Durgavati University, Jabalpur. The test solutions (newly synthesized compounds) were prepared freshly before starting the experiment. All the other reagents were purchased of analytical reagent grade.

Earth-worm *Pheretima postuma* was collected from Jawaharlal Nehru Krishi Vishwavidyalaya (JNKVV), Jabalpur, Madhya Pradesh, India. All earthworms having equal size (5-7cm approximately). The anthelmintic activity was performed *in vitro* using adult *Pheretima postuma* worms owing to their physiological and anatomical resemblance with the intestinal round worms. The bacterial strains are recognized strains and were gained from the Department of Bioscience, Rani Durgavati University, Jabalpur, M.P., India. The microorganism used in the assessment of antibacterial activity was *Escherichia coli.*

Extraction of plant materials

O. sanctum fresh plant was collected and dried and converted into fine powder. The extraction process of powdered leaves were done by addition of 50gm of dried

powder leaves were soaked with 500 ml of alcohol in soxhlet apparatus for 48 hrs, after extraction the solvent was decanted and the residue again soaked with the same solvent for 24 h. Finally the extract was filter through filter paper and collected. Solvent was evaporated by placing the extract on vacuum evaporator.

$CHCl_3$: ethanol (9:1) was taken for extraction of leaves of *Ocimum sanctum* and hexane, ethyl acetate and methanol were used for gradient column chromatography in growing polarity. The presence of ursolic acid was confirmed in fractions by TLC. All fractions, which monitored of ursolic acid, were combined collectively and the solvent was removed under vacuum. A white solid was found after evaporation of the combined fractions containing ursolic acid. Quantity of ursolic acid in the extract was determined by TLC and means of optical densitometry, with identified concentrations of ursolic acid in the samples.

Anthelmintic Activity

Indian adult earthworms (*Pheretima postuma*) were taken for the study of anthelmintic activity. The earthworm which was used for study has similarity in both physiologically and anatomically to the parasites of the intestinal roundworm of the human beings; hence it is suitable to study anthelmintic activity. The earthworms can be categorized into individual groups (each group having three organisms) for each cure at different concentrations. Albendazole the standard drug at three different concentrations of 15, 20 and 25mg/ml with ethanol was made. Likewise, the formulated compound ursolic acid was also formulated at different concentrationof 15, 20 and 25mg/ml as test. To each petri dish three earthworms were taken. The movement of earthworms was examined to each petri dish. The time taken for the worm to lose its movement was considered for paralysis time and the time taken to drop its motility even in the occurrence of outside stimulus (when dipped in warm water at 55ºC) and faded body color was measured for mortality time. Death time and paralysis time of each earthworm in the group was recorded (Bhabani et al., 2009; Tharachand et al., 2015; Nayak et al., 2012).

Antibacterial activity

Disk Diffusion method

The synthesized compound ursolic acid was screened for its antibacterial activity against *Escherichia coli*. A disk diffusion method was used for the assessment of antibacterial action. Six mm filter paper discs were impregnated with 10 mg /20ml and 20 mg/20ml dilutions and were allowed to remain at 37°C till complete diluents evaporation and kept under refrigeration before antibacterial activity was assessed. They were grown routinely overnight in a nutrient broth at 37°C. The antibacterial drug based metal composites and discs of drug were used over each of the culture plates and the turbidity of the test bacteria cultures was used to seed and then it was incubated at 37°C for 18-24 h. The release of drugs into the surrounding agar medium shows growth inhibition of microorganisms and it was evaluated. The zone of growth inhibition around the disk was measured that was used to determine the growth inhibitory effect (Sadal et al., 2012; Joshi et al., 2011; Mishra and Mishra, 2011; Jeba et al., 2013; Tharachand et al., 2015).

Figure 1. Anthelmintic activity of Ursolic acid

Table 1. Anthelmintic activity of the Ursolic acid

Treatment groups	Concentration (mg/ml)	Time of Paralysis (min.)	Time of Death (min.)
Albendazole	15	41.3 ±0.47	50.7 ±0.15
	20	37.8 ±0.35	51.2 ±0.59
	25	25.5+0.42	32.5+0.37
Ursolic acid	15	39.2 ±0.95	47.8 ±0.15
	20	34.3 ±0.38	43.2 ±0.61
	25	22.6+0.43	30.1+0.13

Well Diffusion Method

A loop full of the given test strain was inoculated into 25ml of N-broth (Nutrient Broth) and incubated for 24 h in an incubator at 37 °C in order to activate the bacterial strain. A 100mm diameter petri dish was poured by using Mueller Hinton Agar No.2 media 28–30 ml. The Pour-plate technique was used for inoculation. The activated strain 0.2 ml was inoculated into the medium when it had gained 40–45 °C temperature. A laminar airflow was maintained for the complete procedure of the ditch preparation that helps to maintain strict aseptic and sterile condition. The media was permitted for solidification. A well was prepared after solidification in the media at the middle and then the well was inoculated with 0.1 ml of the synthetic composite (DMF solution). Performance was done under controlled condition for each solvent and for each bacterial strain. Incubation for the plates were maintained for 24 h at 37 °C. The antibacterial activity of synthetic compound can be determined by measuring the inhibition zone which was formed by the compounds against the particular test bacterial strain. For calculating the zone of growth

inhibition of each sample the mean value was obtained for three individual and replicates were used. Antimicrobial activity of complex was investigated by well diffusion method. The Agar plates were pre-inoculated with the test organism and kept for 15 min. In this method well is made with help of sterilized cork on the agar plate. A fixed volume of complex and standard drug was then introduced into the wells. The plates are then incubated at 37°C for 24h. Then the plates were observed for the circumference of the zone of inhibition (Sadal et al., 2012; Joshi et al., 2011; Mishra and Mishra, 2011; Jeba et al., 2013; Tharachand et al., 2015).

Table 2. Antibacterial activities by Disk Diffusion method (zone of microbial growth inhibition in mm)

Strains	Zone of inhibition			
	Tetracycline		Ursolic acid	
	0.5mg/ml	1mg/ml	0.5mg/ml	1mg/ml
Escherichia coli	15.5±0.5	23.6± 1	25.6±2	36.3±0.9

Table *3.* Antibacterial activity by Well Diffusion Method **(**zone of microbial growth inhibition in mm)

Strains	Zone of inhibition (mm)	
	Tetracycline (0.1ml**)**	**Ursolic acid (**0.1ml**)**
Escherichia coli	20.6 ±20	37.3 ±50

Results and discussion

The common cause of parasitic diseases is helminthes or worms. Anthelmintic agents expel and kill the worms from the infected host body but the development of resistance because of the extensive use of these drugs has been found and therefore, there is essential to synthesize, design and develop safe and potent anthelmintic agents. This is proved from the investigational data (Table 1) that, ursolic acid was found to showed significant anthelmintic activity against earthworm *Pheretima postuma* (Table 1 and Figure 1). Screening of the compound was done for activity for complete death and paralysis of worms. The results expose a dose dependent boost in activity of the compound at 15, 20 and 25 mg/ml concentration. The standard drug albendazole, at same concentrations were compared with the result. Disk diffusion method and well diffusion method were used to estimate the results of antibacterial activity of ursolic acid. The in vitro antimicrobial studies (Table 2 and 3) disclose that the ursolic acid was found to be potentially active against microbial species and show superior biocidal effect.

Conclusion

The current study shows the antibacterial and anthelmintic potential of synthetic ursolic acid was potent in their activity against the worms. The results determined that ursolic acid have the power to paralyze and kill the parasitic worms. The compound also has good antibacterial activity that proved the active compounds may be used as potential and safer antibacterial and anthelmintic agents.

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