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EVALUATION OF IN-*VITRO* ANTHELMINTIC POTENTIAL OF UMBELLIFERONE AGAINST *PHERETIMA POSTHUMA*

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ABSTRACT: Objective: The present study was aimed to evaluate the *in-vitro* anthelmintic potential of isolated phytoconstituent umbelliferone against *Pheretima Posthuma* (Indian earthworm). **Methods:** Three different concentrations (10, 20, 30 mg/ml in Dimethyl sulfoxide (DMSO) of umbelliferone were prepared, and six worms of similar type were placed in it. Observations were made for the time taken for paralysis and death of the individual worm. Meantime required for the paralysis (P) in min was noted when no movement of any sort could be observed, except when the worm was shaken vigorously; death time (D) in min was recorded after confirmation with lack of movement when shaken vigorously/ dipped in warm water (50 °C). Piperazine citrate (10 mg/ml) was used as a reference standard. **Results:** Umbelliferone demonstrated paralysis as well as the death of worms, especially at a higher concentration of 30 mg/ml in a shorter time as compared to reference drug Piperazine citrate. **Conclusion:** In the present study, Umbelliferone was tested for its anthelmintic activity against *Pheretima posthuma*. Various concentrations were used in the bioassay, which involved paralysis and death time of the worms. The phytoconstituent showed significant anthelmintic activity.

INTRODUCTION: Helminthiasis, also regarded as a worm infection, is a macro-parasitic disease infecting humans as well as other animals. Helminthiasis is one of the most prevalent diseases in the world, particularly in the areas of poor sanitary control as well as in tropical countries. Also, the main cause of the infection in the developing countries is the lack of adequate sanitary facilities [1]. Anthelmintics or anti-helminthics are regarded as a group of antiparasitic drugs that basically have a mechanism of expelling parasitic worms and other internal parasites from the body by either stunning or killing them without the involvement of the host.

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They can also be regarded as vermicides (those that kill) or vermifuges (those that stun). Anthelmintics are used for the treatment of helminthiasis as well as infected animals [2]. Resistance development became the most prevalent problem with the use of these categories of chemotherapeutic agents; therefore, there is a major problem in the treatment of helminthiasis.

There are several polyherbal formulations in the treatment of helminth infection [3]. Moreover, these categories of drugs are not cost-effective. So, patient acceptability becomes another issue in the consumption of these drugs as a remedy. Taking into consideration the above-mentioned limitations, herbal remedies can be used as safe and effective alternative remedies. Coumarin (1, 2-Benzopyrone or 2H-1-benzopyran-2-one, or phenylpropanoids) and its derivatives are proven to exhibit useful and diverse biological activities [4, 5]. The compound coumarin occurs as secondary metabolites in the various parts of plants as seeds, roots, and leaves of

many plant species [6]. Warfarin, aesculetin (6, 7-dihydroxycoumarin), umbelliferone (7-hydroxy-coumarin), Psoralen, Imperatorin, Herniarin (7-methoxycoumarin) and are some of the naturally occurring coumarin derivatives. Most of the researchers have classified coumarins as simple coumarins (*e.g.*, imperatorin and isopimpinellin), linear pyranocoumarins (*e.g.*, xanthyletin) or angular pyranocoumarins (*e.g.*, seselin), angular furanocoumarins (*e.g.*, angelicin) [7]. However, some researchers used a biogenetic approach based upon the number of nuclear oxygen atoms in classifying coumarin-containing compounds [8].

Coumarin derivatives have various potential therapeutic applications as anti-viral (effect against HIV) virus and antitumor effects [9, 10], antidepressant and Alzheimers [11], anti-bacterial [12, 13], anti-inflammatory [14], anti-coagulants [15], gastro-protectives [16], anti-hyperlipidaemic [17], anti-oxidants [18]. Coumarin derivatives were previously applied as flavoring agents and fixative but are nowadays considered as food adulterants by Food and Drug Administration (FDA) in the United States owing to their potentially harmful side effects such as mild nausea [19], anticancer [20] and metabolic syndrome [21]. Coumarins are reported as hepatoprotectives [22]. Several European countries are using coumarin-type derivatives such as coumarin (1), for the treatment of lymphoedema. Still, the treatment has not been approved for therapeutic purposes in the United States because of hepatotoxic potential. Also, the coumarin derivatives possess several metabolic effects, mainly lipid-lowering effect and anti-diabetic effects [23].

L-phenylalanine is a precursor in a synthesis of a phenylpropanoid derivative umbelliferone, which is produced *via* the shikimic acid pathway. Compound phenylalanine is turned into cinnamic acid, followed by hydroxylation with cinnamate 4-hydroxylase yielding 4-coumaric acid, which is then hydroxylated by hydroxylase to yield 2, 4-dihydroxy-cinnamic acid. Finally, after an intramolecular attack from the hydroxyl group of C2' to the carboxylic acid forms the lactone umbelliferone.

The reference standard used in this assay is piperazine citrate. It acts by causing muscle hyperpolarization due to its GABA mimetic action resulting in the opening of Cl- channels resulting in relaxation and depresses the contractile effect of acetylcholine, thereby causing flaccid paralysis. The worms recover if they are placed in a medium freed from piperazine [24].

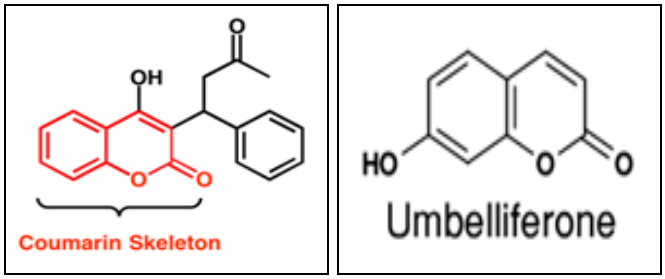

FIG. 1: UMBELLIFERONE STRUCTURE

MATERIALS AND METHODS: Umbelliferone was obtained as a pure compound from Yukka enterprises, Mumbai. Indian earthworm *Pheretima posthuma* (Annelida) were collected from the waterlogged areas of soil; the average size of the earthworm was 6-8 cm. Worms were cleaned with tap water for the removal of the adhering dirt. The procedure for the anthelmintic assay was followed as per the reported procedure [25]. Indian earthworm *viz. Pheretima posthuma* was used due to its anatomical and physiological similarity with the intestinal roundworm parasites of human beings. *Pheretima posthuma* worms are easily available and used as a suitable model for screening of anthelmintic drugs.

Briefly, 20 µl formulations of Umbelliferonein Dimethyl sulfoxide (DMSO) in three different concentrations as 10, 20, and 30 mg/ml were prepared, and standard 10 mg/ml was used in treatment. Test solution and standard drug solutions were freshly prepared just before use, and 'paralysis time' was noted when no movement of any type could be observed except when the worms were vigorously shaken. The 'death time' of worms was noted after confirming that the worms neither moved when dipped in warm water at 50° or when shaken vigorously. A maximum time period of 120 min was allotted for the paralysis as well as the death time of *Pheretima posthuma*, and Umbelliferone treated worms. The experiments were repeated three times. Data were analyzed by one way ANOVA followed by Dennett's test, $P<0.05$ being considered as significant. From the

observations made, a dose-dependent paralytic effect of the compound was seen in **Table 1**.

RESULTS AND DISCUSSION: The anthelmintic activity of Umbelliferone was carried out on *Pheretima Posthuma* (Indian earth warm). Different concentrations of Umbelliferone were used for the present study. The time taken for paralysis and death of earthworms were recorded. There was no paralysis and death in the control group. Paralysis time in standard treatment group was found to be 10.04. ± 1.32 min. Treatment with umbelliferone at all three doses significantly reduced paralysis time in a dose-dependent manner. Umbelliferone in various doses (10, 20, 30 mg/kg) showed paralysis time as 52.64 ± 2.76 (*p<0.5), 32.63 ± 6.677 (**p<0.1) and 22.11 ± 4.84 (***p< 0.001) respectively. This indicates umbelliferone shows a significant reduction in paralysis time at all three dose levels. The effects were seen compared to the standard drug Piperazine citrate (10 mg/ml). Death time in the standard treatment group was found to be 22.06 ± 2.28 min. Treatment with umbelliferone an all three doses significantly reduced death time in a dose-dependent manner. Umbelliferone in various doses (10, 20, 30 mg/kg) showed death time as 108.8 ± 7.16 (*p<0.5), 70.05 ± 13.53 (**p<0.1) and 49.92 ± 10.13 (p< 0.001) respectively. This indicates that Umbelliferone at all three doses significantly reduced both paralysis time and death time, indicating its anthelmintic activity. The recent research on the anthelmintic activity of coumarin substituted derivatives showed potent anthelmintic activities and can be used as a potential lead compound for future discoveries [26].

TABLE: 1 ANTHELMINTIC ACTIVITY OF UMBELLIFERONE

	Dose (mg/ml)	Paralysis time (min)	Death time (min)
Control	-	-	-
Piperazine citrate	10	10.04 ± 1.32###	22.06 ± 2.28###
Umbelliferone	10	52.64 ± 2.76*	108.8 ± 7.156*
Umbelliferone	20	32.63 ± 6.677	70.05 ± 13.53**
Umbelliferone	30	22.11 ± 4.832	49.92 ± 10.13***

CONCLUSION: In conclusion, umbelliferone has got a potent anthelmintic activity against Indian earthworms (*Pheretima Posthuma*).

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CONFLICTS OF INTEREST: None to declare.

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