

Preliminary screening of the bioactive (chemical) compounds in the epigeic earthworm, *Eisenia fetida* cultured in cattle manure

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Abstract- The therapeutic value of earthworms is due to presence of zoo-constituents. Earthworms are also pharmacologically important source of bioactive molecules such as enzymes, protein, peptides, and various other bioactive compounds which exhibit wide range of biological activities are used in the treatment of various diseases especially in Eastern countries. Therefore, the present study was carried out to know the presence of various bioactive compounds in four different solvent extracts of the epigeic earthworm, *Eisenia fetida*. The results of present study revealed that there is a presence of various bioactive compounds like alkaloids, terpenoids, saponin, phlobatannin, lignin and coumarin in different solvent extracts of *Eisenia fetida* earthworm. These bioactive compounds screened in different solvents may be responsible for various biological activities that can be used as pharmaceutically valuable medicines to treat wide range of diseases economically with no side effects.

Keywords- Bioactive compounds, *Eisenia fetida*, Epigeic earthworm and Screening.

I. INTRODUCTION

In ancient times, diseases were treated and rectified by using naturally available products. Most of these natural products/compounds were obtained either from plants or animals. Our prehistoric ancestors lacked the knowledge of these medicinally important components. With the recent development and revolution in Science and Technology, medicinally important bioactive components were identified, isolated and characterized to treat various diseases. Food provides our body with essential nutrients like macro, micro nutrients and non-nutrients like phyto-chemicals and zoo-chemicals both of which are vital to health. Phyto-chemicals are the natural bioactive substances usually obtained from plants, which exhibit

different biological activities. Plants are the source of various drugs used to treat different diseases as they are rich in phyto-chemicals [1]. Phyto-chemicals are classified as primary metabolites, which include proteins, carbohydrates, amino acids, Lipids, nucleic acids etc, and secondary metabolites includes, other chemicals produced by the cells through their metabolic pathways derived from primary metabolites [2,3]. Based on the biosynthetic pathway, secondary metabolites are further divided into three major groups like nitrogen containing compounds, phenolic compounds and terpenes [4,5]. Similarly, zoo-chemicals are also the bioactive substances obtained from animals, which are responsible for different biological activities. Recently, zoo-therapy is also gaining much more importance in pharmacological studies as much research is going on with respect to usage of zoo-chemicals.

Phyto-chemical screening assay is the preliminary and basic technique used to identify the presence or absence of different phyto-chemical compounds in the plant sample, meanwhile for animal sample, it is termed as "Zoo-chemical compounds". Different types of zoo-chemical compounds are present like alkaloids, phenols, flavonoids, steroids, saponin, tannin, anthroquinones, phlobatanin, phytosterol, lignin, carotenoids, quinines, coumarins, etc. These bioactive compounds are functionally protective in nature, which safeguards themselves from bugs, germs, microbes, fungi and other threats [6]. Earthworms are extensively used in Indian and Chinese medicine because of rich diversity of earthworms. There is a history of medicinal use of earthworm in China since almost 4000 years [7] like Folklore medicines claims the successful use of earthworms in the treatment of

various diseases such as cancer, cold, ulcers, fever, inflammation, diarrhea, toothache, dysentery etc. [7]. Wide range of literature is available on the phytochemicals, which are extensively used to study the medicinal importance in almost all the plants, but there is little literature available on the preliminary zoo-chemical analysis of the earthworms. Hence, it is necessary to know and understand the basic zoo-chemical composition of biologically active compounds present in the individual earthworm species. Therefore, the present study was undertaken to study the preliminary screening/ analysis of various zoo-chemicals present in the epigeic earthworm, *Eisenia fetida*.

II. MATERIALS AND METHODS

Preparations of earthworm sample: The epigeic earthworm, *Eisenia fetida* cultured in pure cattle manure were obtained from vermitechnology laboratory maintained at Dept. of Zoology, Karnatak University, Dharwad. The complete worm biomass was powdered as per procedure prescribed by Shradha [8] with slight modifications was used as earthworm sample.

Preparation of solvent extract: Earthworm sample was mixed with four different solvent extracts (Chloroform, Methanol, Ethyl acetate and Distilled water) separately and kept in dark condition for 24 hours. All these four samples were used for analysis of the bioactive compounds.

Qualitative analysis: All four solvent extracts were subjected to preliminary screening (qualitative analysis) of various zoo-chemical compounds such as Alkaloids, Phenolic compounds, Flavonoids, Terpenoids, Steroids, Saponin, Tannin, Anthroquinone, Phlobatannin, Phytosterol, Lignin, Anthrocyenin and Coumarin as per procedure reported by Junaid [9].

III. RESULTS AND DISCUSSION

The results of the present study revealed that different zoo-chemicals were screened in four solvent extracts of the epigeic earthworm, *Eisenia fetida*, and the same is represented in the Table 1. The chloroform extract showed positive results for three bioactive compounds such as alkaloids, terpenoids and phlobatannins, the methanol extract gives positive results for the presence of lignin and coumarin, whereas the presence of

alkaloids, saponin and lignin compounds were obtained in Distilled water extract, but no zoo-chemical compounds were detected in ethyl acetate solvent extract (Table 1). All these positively screened zoo-chemical compounds in the earthworm tissue possess wide range of biological activities, are very much similar to phyto-chemicals obtained from plants. Alkaloids are the nitrogen containing group, which are commonly found in both plants, animals and even in microorganisms. The chloroform, methanol and distilled water extract of earthworm, *Eisenia fetida* showed the positive results for various bioactive compounds like alkaloids, terpenoids, phlobatannins, lignin, coumarin and saponin. The alkaloids are used in defense against predators due to the general toxicity and deterrence capability [10]. Terpenoids are the natural products derived from isoprene units containing oxygen as functional group [11]. Terpenes protect animals, plants and microorganisms from biotic and abiotic stress [12], they also possess anti-insect property, can be used as pesticides to kill insects and other pests without the use of chemical harmful sprays that may cause side effects [13]. Phlobatannin is a novel class of ring isomerized condensed tannins [14], these chemical compounds are known to exhibit analgesic, anti-inflammatory, wound healing [15] and anti-oxidant properties [16]. Lignin is a complex aromatic polymer compound usually found in plant cell wall [17, 18]. In animal nutrition, lignin is an anti-nutritive component of forages as it cannot be readily fermented by rumen microbes [19]. Coumarin is colourless polyphenolic aromatic compound belonging to a group of crystalline oxygenated heterocyclic compound [20, 21]. Biologically coumarin has appetite suppressing activity as it has bitter taste [22]. Saponins are the steroids having bitter taste and foaming properties [23]. These compounds helps in reduction of blood cholesterol level by preventing its re-absorption capacity [24] (Prohp et al. 2012) and exhibit various biological activities [25]. Apart from all above mentioned functions, these biochemical compounds extracted from animals are also known for various biological activities like anti-inflammatory, anti-cancer, anti-diabetic, wound healing, anti-fungal, anti-bacterial activities. Literature revealed that earthworm tissue and their coelomic fluid exhibit various biological activities such as, anti-pyretic, detoxifying, anti-hypertensive, anti-spasmodic, diuretic, anti-asthmatic, anti-ulcer,

spermatocidal, anti-microbial, anti-oxidative, anti-cancer, anti-allergic, anti-inflammatory activities [26-28]. All these biological activities exhibited positively may be because of the presence of different zoo-chemical compounds, which may be either primary or secondary metabolites in the earthworms.

IV. SUMMARY AND CONCLUSION

The results indicate that the four different solvent extracts of the epigeic earthworm, *Eisenia fetida* showed the presence of various zoo-chemical compounds. The chloroform extract showed positive results for alkaloids, terpenoids and phlobatannins. The methanol extract have the presence of lignin and coumarin, whereas distilled water solvent extract showed the presence of alkaloids, saponin and lignin, but no bioactive compounds were detected in ethyl acetate solvent extract. Therefore, chloroform and distilled water solvent extracts are better in screening and isolated of zoo-chemicals in the earthworms followed by the methanol extract. Hence, it is concluded that the presence of all these zoo-chemical compounds primarily screened in the epigeic earthworm, *Eisenia fetida* may be responsible for various biological activities that can be used as pharmacologically important and valuable medicine so as to treat wide range of diseases economically with minimum or no side effects.

ACKNOWLEDGEMENT

Authors extend heartfelt gratitude to all those, who helped directly or indirectly for carrying out this particular work.

FUNDING

The research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Ethical approval: Not applicable.

Conflict of interest: The authors declare that there is no conflict of interest.

Research content: The research content of manuscript is original and has not been published elsewhere.

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Table 1: Presence of various bioactive compounds in the epigeic earthworm, *Eisenia fetida* in four different solvent extracts.

Sl. No.	Bioactive compounds name	Name of the test	Chloroform extract	Methanol extract	Ethyl acetate extract	Distilled water extract
1	Alkaloids	Wagner's test	+	+	-	+
2	Phenolic compounds	Ferric chloride test	-	-	-	-
3	Flavonoids	Shinoda's test	-	-	-	-
4	Terpenoids	-	+	-	-	-
5	Steroid	-	-	-	-	-
6	Saponin	-	-	-	-	+
7	Tannin	Baraymer's test	-	-	-	-
8	Anthroquinone	-	-	-	-	-

9	Phlobatannin	HCl test	+	-	-	-
10	Phytosterol	Salkowski's test	-	-	-	-
11	Lignin	Labat test	-	+	-	+
12	Anthracyanin	HCl test	-	-	-	-
13	Coumarin	NaOH paper test	-	+	-	-

'+' represents positive/present, '-' represents negative/absent