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RESEARCH ARTICLE

Qualitative Analysis of Zoochemicals in *Tubifex*

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ABSTRACT

Animals offer therapeutic benefits due to the presence of zoo compounds. They are the significant source of bioactive molecules, including proteins, peptides, enzymes, carbohydrates, antioxidants and various other bioactive compounds. These molecules display a wide range of biological activities and are used to treat various diseases. The investigations of zoo compounds has attracted significant attention in recent years, because of their potential applications in pharmaceuticals, agriculture, and biotechnology. *Tubifex* are globally distributed aquatic annelids that function as live feed for fish and various aquatic invertebrates due to its nutritional value. This study analyzed zoo compounds present in the extracts of *Tubifex* from low polar hexane and high polar aqueous. The presence of alkaloids, tannins, saponins, steroids, terpenoids, flavonoids, phenolic compounds, and cardiac glycosides in *Tubifex* extract was

confirmed. The bioactive compounds assessed in these two solvents may play a role in various biological activities relevant to biomedical application.

Keywords: Aqueous extracts, biomedical application, hexane extract, *Tubifex* and zoo compounds

INTRODUCTION

In ancient times, diseases were addressed and remedied through the use of naturally occurring substances. Many of these natural products or compounds were derived from either plant or animal sources. Prehistoric ancestors did not possess knowledge of these medicinally significant components. Recent advancements in science and technology have led to the identification, isolation and characterization of bioactive components with medicinal significance for the treatment of various diseases. Food provides essential nutrients to the body, including macronutrients, micronutrients and non-nutrients such as phytochemicals and zoo-chemicals, all of which are crucial for health (1). Metabolites are naturally occurring bioactive compounds derived from plants and serve as a source of numerous pharmaceuticals for the treatment of various diseases due to their widely distribution(2,3). Metabolites are categorized into primary metabolites, encompassing proteins, carbohydrates, amino acids, lipids and nucleic acids, and secondary metabolites, which consist of additional compounds synthesized by cells via metabolic pathways originating from primary metabolites. Secondary metabolites are classified into three primary categories based on their biosynthetic pathways: nitrogen-containing compounds, phenolic compounds and terpenes(4,5). Zoo chemicals are bioactive substances derived from animals that contribute to various biological activities. Zoo therapy has recently become significant in pharmacological studies, with research focused on the application of zoo chemicals for the treatment of various diseases, including anti-cancerous activity, anti-microbial activity, anti-inflammatory, anti-diabetic activity, anti- oxidative activity, fever, diarrhea, dysentery, biomarkers and probiotics(6–10). Researchers have extensively studied phytochemicals and their therapeutic value in a variety of plants, but little is known about the zoo chemical analysis of annelids. So, it is extremely significant to know the basic zoo chemical constituents of the *Tubifex* extracts. The current study examines the presence of bioactive compounds in *Tubifex*. This preliminary screening establishes a basis for subsequent in-depth investigations, potentially resulting in the identification of new therapeutic agents and other significant bioactive compounds. This research enhances the understanding of annelid biochemistry and highlights *Tubifex* as a significant source of bioactive compounds with various applications.

MATERIALS AND METHODS

Preparation of sample

The *Tubifex* that had been freeze-dried were purchased from an aquarium retailer specifically for the purpose of the study, and then they were powdered using a mortar and pestle.

Preparation of Solvent extract

5g of Freeze-dried *Tubifex* powder was separately mixed with two solvents of differing polarity (hexane and distilled water) of ratio 1:10 and the mixture was shaken occasionally under dark environment for 72 hours. The extracts were filtered using Whatman filter paper no. 2 on a Buchner funnel and were subsequently utilised for the analysis of zoo compounds.

Qualitative analysis

Qualitative tests for alkaloids, steroids, tannins, saponins, terpenoids, flavonoids, cardiac glycosides, phenolic compounds, aromatic acids and xanthoprotein were conducted by following the methodology described by Harborne, 1998.

RESULTS AND DISCUSSION

The zoo chemical of two extract of the freeze-dried *Tubifex* were analyzed. The polar compound hexane is utilized due to its ability to dissolve an array of nonpolar compounds. The results obtained showed the evidence of zoo compound from the hexane extract. In this analysis Steroid, Tannins, Saponins, Terpenoid, Cardiac Glycosides, Phenolic compound and Xanthoprotein were present, whereas alkaloid and aromatic acid were absent (Table 1). The Eco friendly solvent aqueous is polar in nature that has been used for the extraction. Table 2 indicates the zoo chemicals present in the aqueous extract. Alkaloids, Steroid, Tannin, Saponins, Flavonoid, Terpenoid, Cardiac Glycoside, Phenolic compound were present while Aromatic acid and Xanthoprotein were absent in the extract. This study revealed that, both solvents were effective in the extraction of zoo constituents. These positively screened biochemical compounds in *Tubifex* extract exhibited a wide range of biological activities, and they closely match secondary metabolites found in various medicinal plants. This highlights the potential for *Tubifex* extract to serve as a natural source for the discovery of new therapeutic agents in drug development. Alkaloids are one of the largest groups of natural products containing nitrogen. This compound exhibit pharmacological activities such as antioxidant property, anticancer activity, antimicrobial activity, amoebicidal activity, cytotoxicity activity, muscle relaxant, analegisic property, antimalarial activity, antituberculosis activity etc(12,13). Steroids are four ring structure present in both the extract and has the following activity of anti-inflammatory, immunosuppressive, anti-hepatotoxicity and anti-tumour activity(14,15). The polyphenolic compounds like tannins has Anti-oxidant, anti-inflammatory, anticancer antimicrobial properties, along with their role in the prevention of cardiovascular, neuroprotective and metabolic diseases (16). According to Desai et al. (2009), saponins express various effects on animal cells, fungi and bacteria; however, limited research has focused on their role in plant cells and they are recognised for their antimicrobial properties, their ability to inhibit mould growth, and their role in safeguarding plants against insect infestations. Researchers reported that the flavonoids are one of the most abundant and widespread secondary metabolites groups, are valuable to humans for their effects such as anti-diabetic effect, antiosteoportic effect, cardioprotective effect, neuroprotective effect, antioxidant effect and some other effects like asthma, skin damage and erythema (18–21). Terpenoids are the compound derived from isoprene group and their pharmacodynamics are antimalarial drug, anti-melanoma, chemopreventive, anti-inflammatory and anti-arthritic activity (22). Organic compound like cardiac glycosides bind to and inhibit Na^+/K^+-ATPase and also involved in cancer therapy (23). Aromatic compound phenol is directly attached to the benzene ring were used in anti-inflammatory activity (24). In addition, the phenolic compound possess antibacterial, antiprotozoal, antifungal, antitumor, estrogenic, antidiabetic, or antithrombotic agents (25).Similar to plants, animals are increasingly recognized as an alternative source for bioactive compounds. Numerous researchers have worked to identify animals that produce bioactive compounds and have gained success(26–28). Hence this work would lead to the development of new therapeutics and holds the development of drug discovery and treatment of various diseases.

CONCLUSION

The study examines the composition of various chemical groups in the hexane and aqueous extracts of the *Tubifex*. The results indicated the presence of various chemicals in the tested animal. Hexane extracts contained steroids, tannins, saponins, flavonoids, terpenoids, cardiac glycosides, phenolic compounds and xantho proteins while Aqueous extracts contained alkaloids, steroids, tannins, saponins, flavonoids, terpenoids, cardiac glycosides and phenolic compound. This indicates a distinct biochemical profile of the animal, which may influence their respective medicinal properties and uses. Further research is needed to explore the biological activities of these compounds and their potential applications in traditional medicine.

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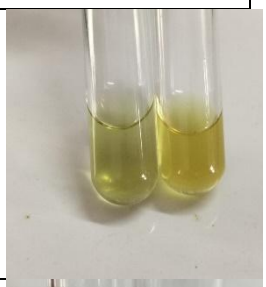
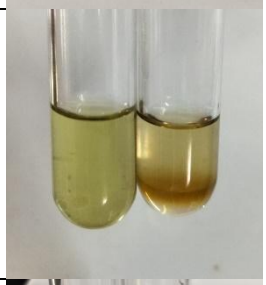
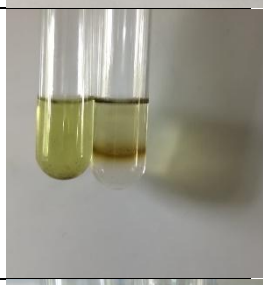
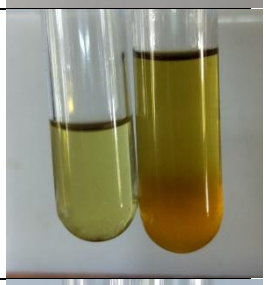
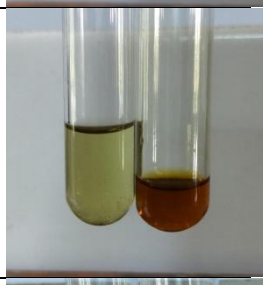
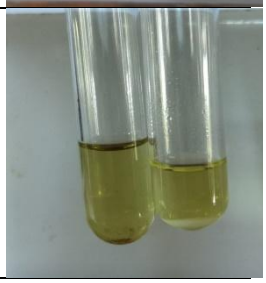
Table 1: Qualitative analysis of bioactive compounds in the Hexane Extract of freeze-dried *Tubifex*.

Sl. No	BIOCHEMICALS	NAME OF THE TEST	OBSERVATION	EXTRACT(PRESENT/ABSENT)	FIGURE
1.	Alkaloids	Mayer's Test	White turbidity or precipitate does not develops	Absent	
2.	Steroids	Salkawski Test	Appearance of redcolor in the lower chloroform layer	Present	
3.	Tannins	Lead Acetate Test	Appearance of White precipitation	Present	
4.	Saponins	Solubility Test	Foamy lather develops.	Present	

5.	Flavanoids	Alkaline reagent Test	Intense colour disappear	Present	
6.	Terpenoids	Libermann-Burchard Test	Inter-phase with the reddish brown coloration is formed.	Present	
7.	Cardiac Glycosides	Keller-Killiani Test	Brown ring appears.	Present	
8.	Phenolic compound	Ferric Chloride Test	Intense colour develops.	Present	
9.	Aromatic Acid	Bicarbonate Test	Formation of brick effervescence not occurs.	Absent	

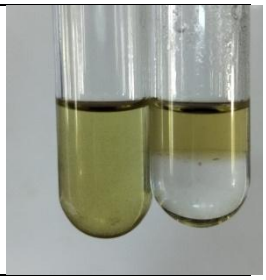
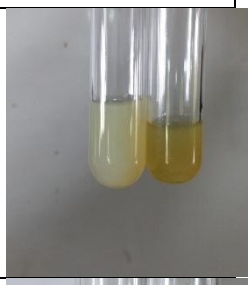
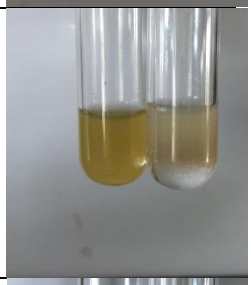
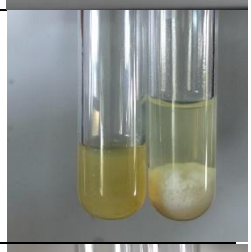
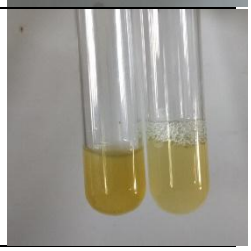
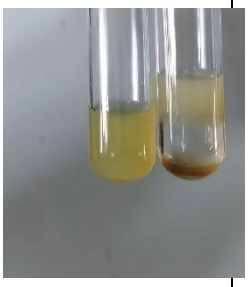
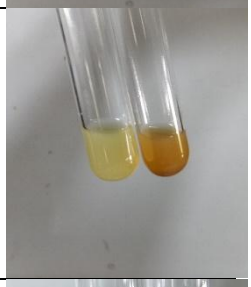
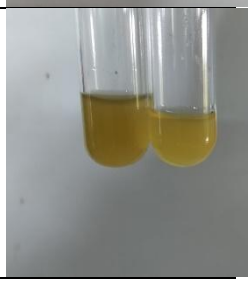
10.	Xanthoprotein	Xanthoprotein test	Yellowish orange precipitation appears.	Present	

Table 2: Qualitative analysis of bioactive compounds in the Aqueous Extract of freeze-dried *Tubifex*sps.

Sl. no	BIOCHEMICALS	NAME OF THE TEST	OBSERVATION	EXTRACT(PRESENT/ABSENT)	FIGURE
1.	Alkaloids	Mayer's Test	White turbidity or precipitate develops	Present	
2.	Steroids	Salkawski Test	Appearance of redcolor in the lower chloroform layer	Present	
3.	Tannins	Lead Acetate Test	Appearance of White precipitation	Present	
4.	Saponins	Solubility Test	Foamy lather develops.	Present	

5.	Flavanoids	Alkaline reagent Test	Intense colour disappear	Present	
6.	Terpenoids	Libermann -Burchard Test	Inter-phase with the reddish brown coloration is formed.	Present	
7.	Cardiac Glycosides	Keller-Killiani Test	Brown ring appears.	Present	
8.	Pheolic compound	Ferric Chloride Test	Intense colour develops.	Present	
9.	Aromatic Acid	Bicarbonat e Test	Formation of brick effervescence not occur.	Absent	

10.	Xanthoprotein	Xanthoproteic test	Yellowish orange precipitation does not appears.	Absent