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Total phenol content, Insecticidal and Amylase inhibitory efficacy of *Heterodermia leucomela* (L).

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ABSTRACT

The present study was undertaken to investigate total phenol content, insecticidal and amylase inhibitory activity of methanol extract of a macrolichen Heterodermia leucomela (L) collected from Bhadra wildlife sanctuary, Karnataka. Total phenol content was estimated by Folin-Ciocalteu reagent method. Insecticidal activity of different concentrations of extract was determined by larvicidal effect on 2nd and 3rd instar larvae of the mosquito Aedes aegypti. Amylase inhibitory activity of the extract was tested against Diastase (Fungal). A marked concentration dependent mortality of larvae was observed. Among larvae, 2nd instar larvae were shown to be more susceptible to extract than 3rd instar larvae. The mortality of 2nd and 3rd instar larvae was recorded 100% at extract concentration 1.5mg/ml and 2mg/ml respectively. The extract showed dose dependent inhibition of amylase. The highest inhibition of amylase was 38.57% at extract concentration 25mg/ml. Thin layer chromatography showed Atranorin and Salazinic acid. Total phenol content was 50.20mg Gallic acid equivalents/g dry weight of extract. The larvicidal and amylase inhibitory effect of extract could be due to the presence of phenolic secondary metabolites.

Key words: *Heterodermia leucomela* (L), Bhadra wildlife sanctuary, *Aedes aegypti*, Larval mortality, Amylase.

INTRODUCTION

Lichens and their products have been used traditionally as medicines for centuries and still hold considerable interest as alternative treatments in various parts of the world. India has rich lichen diversity, contributing nearly 15% of the 13,500 species of lichens so far recorded in the world [1]. In various systems of traditional medicine worldwide, including the Indian system of medicine, the lichen species have shown to cure dyspepsia, bleeding piles, bronchitis, scabies,

stomach disorders, and many disorders of blood and heart [2-4]. *Heterodermia leucomela* (L.) is a foliose lichen (Figure 1) belonging to the family Physciaceae. The thallus is mineral grey, loosely attached to the substratum, lobes dichotomously branched, ascending, revolute, upper surface uneven, medulla white very thin, marginal rhizins present, black in colour. Atranorin and Salazinic acid are present [5]. In Sikkim, *H. leucomela* is used in wound healing [2]. Broad spectrum antifungal properties of water soluble fraction was demonstrated against fungi such as *Fusarium oxysporum*, *Curvularia lunata* etc [6]. Ethanol extract of *H. leucomela* showed marked antimycobacteral properties against *Mycobacterium tuberculosis* H37Rv and H37Ra strains [7]. Literature survey on *H. leucomela* revealed that many biological activities including insecticidal and amylase inhibitory activity remain unexplored. In the present study, we have investigated total phenol content, insecticidal and amylase inhibitory activity of methanol extract of *H. leucomela* collected from Bhadra wildlife sanctuary, Karnataka.

Figure 1: *Heterodermia leucomela* (L.)

MATERIALS AND METHODS

Collection and identification of Lichen material

The lichen *H. leucomela*, growing on the bark of trees of Bhadra wildlife sanctuary, Karnataka, was collected during February 2011. The voucher specimen of lichen (Voucher no. PK/MB/001) was deposited in the Department of Microbiology, SRNMN College of Applied Sciences, Shivamogga for future reference. The lichen material was identified based on morphological, anatomical and color tests. Thin layer chromatography in solvent A (180ml toluene: 60ml 1,4, dioxine: 8 ml acetic acid) was performed to detect secondary metabolites [5,8,9].

Extraction of powdered lichen material

For extraction, 20g of powdered lichen material was added to 100 ml methanol, sonicated for 30 minutes and left at room temperature overnight. The extract was filtered over Whatman No 1 filter paper and the filtrate was concentrated under reduced pressure to pasty mass [10].

Total phenol content of extract

Total phenol content of methanol extract of *E. cirrhatum* was determined by Folin-Ciocalteu method. A dilute concentration of the lichen extract (0.5ml) was mixed with 0.5ml of 1:1 diluted Folin-Ciocalteu reagent and 4ml of sodium carbonate (1M). The mixtures were allowed to stand for 15 minutes and the total phenol content was determined colorimetrically at 765nm. A

standard curve was prepared by using an increasing concentration of Gallic acid in methanol. Total phenol value was expressed in terms of Gallic acid equivalent [11].

Screening for Insecticidal activity

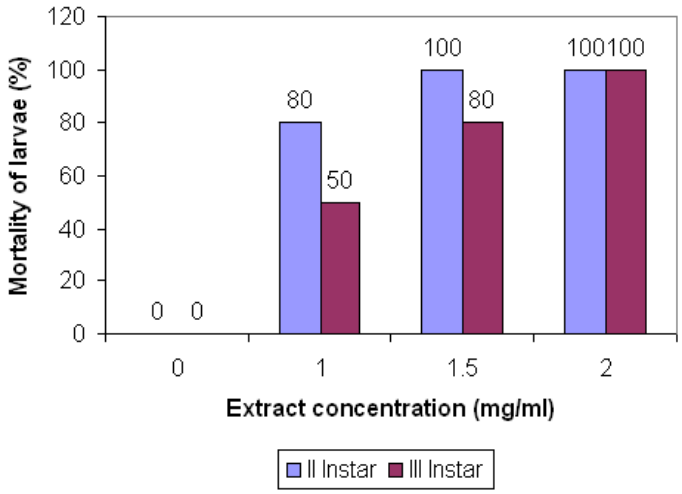
The insecticidal efficacy of different concentrations of methanol extract of lichen was determined against 2nd and 3rd instar larvae of *Aedes aegypti*. Twenty larvae were placed separately into beakers containing different concentrations of methanol extract. A beaker containing 10% DMSO (without extract) serves as control. The larvicidal effect of the extracts was determined by counting the number of dead larvae after 24 hours. Dead larvae were identified when they failed to move after probing with a needle in siphon or cervical region. The test was repeated thrice and the percentage of larval mortality for each concentration of extract was calculated [10].

Screening amylase inhibitory activity of lichen extract

The inhibitory activity of different concentrations of extract of the lichen was determined against fungal amylase (Diastase (Fungal) 3240, Lobachemie Laboratory reagents and fine chemicals, Mumbai) by following the method Jayasri *et al* [12] with minor modifications. The enzyme (0.5%) was prepared in phosphate buffer (pH 6.8). Briefly, 500µl of different concentrations of lichen extract and 500µl of 0.1M phosphate buffer (pH 6.8) containing amylase were incubated at 25°C for 10 min. After preincubation, 500µl of a 1% starch solution in 0.1M phosphate buffer (pH 6.8) was added to each tube and further incubated at 25°C for 10 min. The reaction was stopped by addition of 1ml of dinitrosalicylic acid reagent. The same was performed for control where extract was replaced with buffer. The test tubes were placed in a boiling water bath for 10 min and cooled. To each tube, 10ml of distilled water was added and the absorbance was measured at 540 nm. The percentage (%) inhibition was calculated using formula: % Inhibition = [A_{540}Control - A_{540} Extract / A_{540}Control] x100

RESULTS

Figure 2: Larvicidal efficacy of extract of H. leucomela

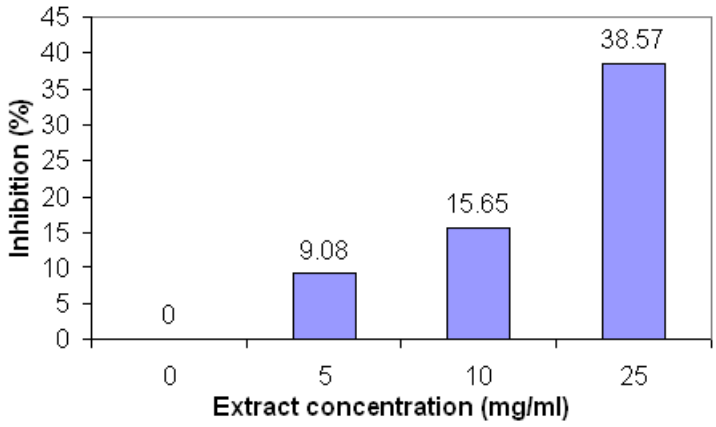


Thin layer chromatography revealed the presence of secondary metabolites namely Atranorin and Salazinic acid. Total phenol content was found to be 50.20mg Gallic acid equivalents/g dry weight of extract. The insecticidal efficacy of different concentrations of lichen extract was evaluated against 2nd and 3rd instar larvae of *A. aegypti*. The mortality of the larvae by the extract was found to be concentration dependent. Among larvae, 2nd instar larvae were found to be more susceptible when compared to 3rd instar larvae. The mortality of 2nd instar larvae was recorded

100% at extract concentration of 1.5mg/ml and 2mg/ml whereas 100% mortality of 3rd instar larvae was observed at extract concentration of 2mg/ml (Figure 2).

The amylase inhibitory activity of the lichen extract was determined against Diastase (amylase) from fungal source. The extract caused a dose dependent inhibition of amylase activity. The highest inhibition of amylase (38.57%) was observed at extract concentration 25mg/ml (Figure 3).

Figure 3: Amylase inhibitory efficacy of extract of *H. leucomela*



DISCUSSION

Lichens produce secondary metabolites that are unique when compared to metabolites of higher plants. Most known lichen substances are phenolic compounds (e.g., orcinol and β-orcinol), anthraquinones (e.g., parietin), dibenzofurans (e.g., usnic acid), depsides (e.g., barbatic acid), depsidones (e.g., salazinic acid), depsones (e.g., picrolichenic acid), γ-lactones (e.g., protolichesterinic acid, nephrosterinic acid), and pulvinic acid derivatives (e.g., vulpinic acid). Lichens had to evolve diverse biosynthetic pathways to produce such complex arrays of secondary metabolites. The polyketide biosynthetic pathway appears to be responsible (in whole or in part) for most of the classes of compounds mentioned before, whereas pulvinic acids are shikimate derivatives, and the abundance of di- and triterpenoids found in lichens are formed via the mevalonate pathway [13-15]. Lichen substances exhibit a great diversity of biological effects, including antimicrobial, antiinflammatory, analgesic, antipyretic, and antiproliferative and cytotoxic activities, and there has been a growing interest in the pharmaceutical properties of compounds derived from lichens [16].

It is estimated that every year at least 500 million people in the world suffer from one or the other tropical diseases such as malaria, lymphatic filariasis, dengue, trypanosomiasis, leishmaniasis etc. Chikungunya, a serious mosquito borne epidemic has gained momentum in India. These diseases not only cause high levels of morbidity and mortality, but also inflict great economic loss and social disruption on developing countries such as India. Due to the lack of awareness among people, early detection and complete treatment of these diseases are very difficult. Consequently several chemical methods are available for the interruption of their transmission which has been limited by logistic problems, development of resistance, high cost etc. So the most effective and easiest approach to control these diseases requires interruption of the life cycle of the vectors by applying larvicides to their breeding places. Further, toxicity of synthetic insecticides towards non-target animals and environment has been widely observed and recognized. To avoid the bioaccumulation and induction of malignancy in non-target animals, a safe and more congenial method of vector control by natural and cheaper means of using plants

as insecticides became popular. Further more, the development of resistance by pests and vectors against botanicals have not been reported so far [17-19]. It is observed that the carbohydrates, saponins, phytosterols, phenols, flavonoids and tannins are having mosquito larvicidal activity. Prenylated xanthones, tetracyclic phenols and saponins are reported to be effective in controlling mosquito *A. aegypti*, the vector of yellow fever [20-21]. In this study, the larvicidal activity of lichen extract could be mainly due to the presence of phenolic constituents and other secondary metabolites.

Diabetes mellitus is an endocrinal chronic disease caused by altered carbohydrate metabolism and characterized by elevated blood glucose levels. There are two main types of diabetes, type I and type II that they affect more than 200 million people worldwide. The most prevalent form of diabetes is non-insulin dependent diabetes mellitus (type II) accounting for 90% of cases throughout the world. The control of hyperglycemia is critical in the management of diabetes because in long term, acute and chronic complications can occur if blood glucose concentration is not kept in normal levels [22]. Pancreatic α-amylase is an enzyme that hydrolyzes the starch to oligosaccharides and maltose in small intestine. Membrane bound α-glucosidase hydrolyzes di- and oligosaccharides to glucose. Inhibition of these tow enzymes reduces the rate of starch digestion and result in decrease in post-prandial blood glucose levels especially in diabetic patients [23]. One therapeutic approach to decrease the hyperglycemia, especially after a meal, is to retard and reduce the digestion and absorption of ingested carbohydrates through the inhibition of carbohydrate hydrolyzing enzymes. One such drug is Acarbose which inhibits alpha-glucosidase enzymes in the brush border of the small intestines and pancreatic alpha-amylase. Other drugs that belong to this class are miglitol and voglibose. Acarbose reduces post-prandial hyperglycemia and is used to treat type-2 diabetes [24]. However, these drugs are known to have gastrointestinal side effects such as abdominal pain, flatulence and diarrhea in the patients [25]. Therefore, it becomes necessary to identify the amylase inhibitors from natural sources having lesser side-effects. Herbal medicines are getting more importance in the treatment of diabetes as they are free from side effects and less expensive when compared to synthetic hypoglycemic agents. Ethnobotanical studies of traditional herbal remedies used for diabetes have identified a number of plants with hypoglycemic activity. Numerous medicinal plants and their formulations are used for treating diabetes in the traditional system of medicine as well as in ethnomedicinal practices [26]. The inhibition of amylase by the lichen extract could be mainly due to the presence of phenolic constituents and other secondary metabolites.

The Folin-Ciocalteu method is widely used to determine phenol content in extracts. It is accepted that the F-C reagent contains phosphomolybdic/phosphotungstic acid complexes and the chemistry behind the FC method counts on the transfer of electrons in alkaline medium from phenolic compounds and other reducing species to molybdenum, forming blue complexes that can be monitored spectrophotometrically at 750–765 nm. The phenolic compounds react with FCR only under basic conditions. The total phenol assay by FC method is convenient, simple, and reproducible [27-29]. In this study, the total phenol content of the extract is found to be 50.20mg Gallic acid equivalents/g dry weight of extract.

CONCLUSION

The larvicidal effect of methanol extract of *H. leucomela* is suggestive that the lichen could be a suitable source for bioactive compounds active against insect vectors such as *Aedes aegytpi* etc to control arboviral diseases such as chickungunya, dengue etc. A marked inhibition of amylase by the extract could be exploited in the development of agents active against pancreatic amylase which is involved in the rise of postprandial glucose level and hence the lichen could possibly

used against diabetes. The observed activity could be because of the phenolic content and other secondary metabolites in the extract.

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