Some Natural Plant Extracts can be used as Leishmania Stains

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Abstract

Visceral leishmaniasis is a chronic disease caused by Leishmania donovani. Staining method is an effective way for identification of parasites by using synthetic or natural dyes. In this study Morus nigra, Punica granatum and Quercus infectoria extracts were used as natural dyes to investigate their staining activity to stain L. donovani promastigote in comparison with geimsa stain. Results: the three extracts showed a good staining ability, M.nigra was the best followed by P. granatum and Q. infectoria. Conclusion: according to the results, dying with natural dyes is cheap, safe and could be alternative for synthetic dyes.

Keywords: Natural dyes, Synthetic dyes, L. donovani, Extrac.

Introduction

Leishmania donovani is a causative agent of the chronic disease visceral leishmaniasis complex. L. donovani is distributed over through North Africa, Asia, Southern Europe and Latin America. Infection with leishmaniasis is transmitted by the bite of phlebotomine sand fly species [1, 2]. Stains or dyes were used to add color to plant tissues, animal tissues and microbes to make them optically distinguished [3]. Staining procedure is the more accurate identification of parasites in parasitological laboratory by using synthetic or natural dyes (hematoxyline, eosin, lugols iodine, carmine, giemsa Romanowsky stain, etc.)[4]. Giemsa stain is recommended for revelation of many parasites including L. donovani [5].

The technique of dyeing is as ancient as human civilisation. Some weavers, knitters and craft use natural dye as a specific feature of their work [7]. Natural dyes are obtained from extracts of flowers, leaves, roots and seeds of some plants by boiling, scraping powdering and mixing them with other materials to get the perfect color. Recently the use of natural dyes has decreased due to the development of synthetic dyes [8]. Synthetic dyes are simpler, easier to use and brighter color [9], but their applications are bounded due to their hazards to human [10]. Almost all synthetic dyes are imported and expensive [11], they synthesized from petrochemical sources and some of them contain carcinogenic amines[12]. Hence, the use of non-allergic, eco-friendly and non-carcinogenic natural dyes has again gained interest to avoid some hazardous synthetic dyes[13]. A perfect biological stain must be effective, cheap, and less toxic and the source must be available [14]. In this research black mulberry (Morus nigra), pomegranate (Punica granatum) and gall oak (Quercus infectoria) extracts have been used as natural dyes for staining L. donovani promastigote in comparison with the recommended stain (Giemsa stain).

Materials and Methods

Preparation of Plant Dye Extract

The Black Mulberry

Approximately 500 g of black mulberry fruits were procured from the local market Baghdad/Iraq then were mashed in a juice machine (MK-8710, National, Japan). The extract was purified by two steps filtration process. Initially, the extract was filtered through wire mesh followed by filtration using what man paper. The pH of the filtration was measured by pH paper (pH 2.8) then heated to just below boiling point to concentrate the dye then cooled and stored at 4°C until use.
The Pomegranate

Fruits were purchased from local market, washed with water and wiped completely dry, then fruits were cut in two halves and the juice was immediately extracted using a hand operated juice extractor/mechanical press.

The obtained raw juice was filtered through wire mesh followed by filtration using what man paper. PH of the filtration was measured (pH 3.5) then heated to just below boiling point, then cooled and stored at 4°C until use.

The Gall Oak

About 25g of dried gall oak powder was purchased from local market.

After thorough cleaning and removal of foreign materials, powder was soaked in 100ml distilled water for 14 hours, and then filtered through wire mesh followed by filtration using what man paper. The pH of the filtration was measured (pH 4.2) then heated to just below boiling point then cooled and stored at 4°C until use.

Slides Preparation

Leishmania donovani strain was obtained from department of biology, Al-Mustansiriyah University. Put one drop from L. donovani culture on clean dry glass slides and making thin smearing, left to dry then fixed with put one drop from absolute methanol and left to dry till staining.

Staining Methods

Five slides prepare for each plant extract. Few drops of the dye were placed on the glass slide for 20 minutes and five slides colored with giemsa stain in order to compare it with the plant extracts used in this study. Then washed with distilled water and left to dry, then all slides were examined under light microscope.

Result and Discussion

The potential natural extracts as staining agent for L. donovani promastigote was investigated in this study. The parasite was colored with the dyes of the extractions; this indicated that, these extractions were able to penetrate the cell membrane of the parasite.

When comparison with giemsa stain Figure1 the parasite appeared with bright pink color when stained with black mulberry Figure 2, while it appeared with light pink color when it is stained with pomegranate Figure 3, and with light brown color when stained with gall oak Figure 4.

![Fig.1: Stained L. donovani promastegot by giemsa stain (100X)](image1)

![Fig.2: Stained L. donovani promastegot by black mulberry (100X)](image2)
The dying degree was varied between the extractions, black mulberry was more efficient than pomegranate and gall oak in staining the parasite, it was an excellent herbal dye with the ability to clearly stain in some cases better than giemsa stain, this result was Similarly noticed by [15] when the juice of black mulberry was used with red beet for staining parasites and showed that these dyes are almost like Carmen stain. Also [16] applied black mulberry extract in staining animal tissue; they proved that this dye method can be an alternative to current chemical staining method.

Black mulberry is a good source of phenolic including anthocyanins and other flavoids and carotenoids [17], pomegranate also contains anthocyanins such as cuanidin, delphinidin and pelargonidin glycosides [18]. Anthocyanins pigments molecules are responsible for the blue, red and purple colors for black mulberry [19] and the red color for pomegranate juice [18]. The main content of gall oak is tannins (gallic acid, ellagic acid, tannic acid and their derivatives) [20, 21] tannins have been used in dyeing cotton fiber since ancient times [22]. The dyestuff in tannin of gall oak is ellagic acid [23] which displays dying features because of its auxochrom group together with other chromogen group [24]. All extracts in this study showed an acidic condition when examined by pH paper. The ability of the dyes to stain is determined by their pH value; acidic structures are stained by basic dyes while basic structures are stained by acidic dyes[25], this mean the dyeing with high fastness can be achieved in acidic pH range[26]; this could be a reason explaining why the extracts were able to stain the parasite.

Many previous studies used natural plant dyes for staining various parasites; henna, alizarin and curcuma were applied as a natural herbal dyes for staining Fasciola hepatica[27], also Mohanad et.al.,[28] stained helminth parasites by Beta vulgaris L. extract; in other study nematodes (Trichuris trichiura and Ascaris lumricoides) were stained with Hibiscus rosa-sinensis L. and Beta vulgaris extracts[29]. Recently in new studies artificial food colors were utilized for staining method; Mohammed et.al, [30] referred to the possibility of using artificial...
food colors for staining L. donovani food colors also employed in staining root-knot nematode[31]. Using natural dyes are not only gave a perfect results but also provide a safe alternative to the harmful, toxic and potentially carcinogenic chemicals used in usual staining techniques.

Conclusion

The uncostly and safely natural plants Morus nigra, Punica granatum and Quercus infectoria are possess the potential to replace the conventional dye (giemsa stain) in staining Leishmania donovani promastigote.

References


