

## STAINING OF *LEISHMANIA DONOVANI* PROMASTIGOTES BY NATURAL FLOWER DYES

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Article received 5.5.2018, Revised 24.5.2018, Accepted 1.6.2018

### ABSTRACT:

There are many synthetic dyes used to stain leishmania parasite for the purpose of diagnosis and the most famous are geimsa stain and leishman stain. Due to the damage caused by the use of synthetic dyes, including large environmental pollution and harmful toxic side effects this study came for the purpose of obtaining alternative dyes for the staining of the parasite from natural sources available, cheap and easy to obtain. The water extract of kujarat flowers *Hibiscus sabdariffa* and the alcoholic extract of Damask rose *Rosadamascena* and China rose *Hibiscus rosa sinensis* were prepared and used in staining the *Leishmania donovani* promastigotes and compared with geimsa stain. The three natural flower extracts gave a good capability in staining the promastigote of *Leishmania donovani*.

Key words: leishmania parasite, stain, kujarat flowers, Damask rose, geimsa

### INTRODUCTION

Leishmaniasis is a world health problem and a major killer in endemic countries. It exists in three clinical forms: cutaneous leishmaniasis (CL); mucocutaneous (MCL) and visceral leishmaniasis (VL). Human visceral leishmaniasis a severe disease. In Recent years witnessed extraordinary progress in diagnosing *Leishmania* infection. The main challenge in these trials has been to reach the standard test in order to establish effective programs to control and eradicate the disease, Laboratory diagnosis of VL includes microscopic examination and culture from sufficient samples, serological tests, and detection of parasite DNA (Elmahallawy *et al.*, 2012). Microscopic examination is the common method for detecting visceral leishmaniasis and the dyes that are frequently used for viewing within different microscopes are either synthetic dyes or natural dyes which are used to identify the parasite stage by detecting specific morphological feature and details of internal structure (Herwaldt,1999: Niranjana *et al.*, 2015).

Giemsa stain is one of the synthetic dyes used to differentiate parasites (Elmahallawy *et al.*,2012: Barcia, 2007). It was used to detect various parasites including blood pathogen like (*L. donovani*, *Plasmodium*, *Trypanosoma spp.* And *Microfilaria* in thick and thin blood films, *Toxoplasma gondii* in the imprints of brain, lung and other tissues and *Entamoeba histolytica* and *Giardia lamblia*, in the imprints of gastrointestinal biopsy (Barcia, 2007).

Synthetic dyes costly material and some of them can cause an allergic response, while some are cancer-causing in nature (Ratna and Padhi,2012). Synthetic food colors are also used in the diagnosis of leishmania parasite because they are cheaper, stable in heat and available (Mohammed

*et al.*, 2016). But they are still considered as synthetic materials, so many researchers have studied using of natural dyes in staining various parasites because they are an effective and safe alternative as well as being cheap and easy to get in the laboratory (Herwaldt,1999: Cheng *et al.*, 2014). some dyes were obtained from the extracts of many garden flowers mostly by boiling, scraping, powdering and mixing with other materials to get desired color (Daryani *et al.*, 2011). *Hibiscus sabdariffa* (Roselle) is a shrub belongs to Malvaceae family, it is developed in numerous countries of the world, and Iraq is one of these countries it is known as kujarat. The water extract of kujarat flower is red and its taste is acidic (Hashim,2006). Parts of the flower are used to make a popular drink in Egypt called Karkade also it is used to make medicine treating loss of appetite, colds (Mahadevan *et al.*, 2009).Tea mixtures are said to bring down fevers and hypertension, relieve coughs, increase urination and kill bacteria (Essiett and Iwok, 2014). Also previous examinations utilized watery extract of kujarat in food industry as a flavoring to improve the smell, flavor of food and drinks(Mahadevan *et al.*, 2009). Others used its watery extract to stain the fungi, plants and blood film (Hashim,2006).

The China rose, *Hibiscus rosa-sinensis*, is a species of Malvaceae family and develops as an evergreen herbaceous plantit's a common flower found in many parts of the world (Kumar and Singh, 2002). Flowers of *Hibiscus rosa-sinensis* are edible and can be made into a kind of pickle or used in salads in the Pacific Islands or as a purple dye for coloring foods such as preserved fruit and cooked vegetables. Flower preparations are used for hair care. Alsois used primarily for respiratory problems, skin disorders and to lower fevers (Essiett and Iwok,2014).

*Rosa damascena* commonly known as Damask rose, the most important species of Rosaceae family flowers. *R. damascena* is a decorative plant grown for their flowers in gardens around the world. Their colorful flowers range from white through yellow, pink, purple and red (Niranjan *et al.*, 2015). *R. damascena* is mainly known for its fragrant smell and beside several pharmacological properties including anti-viral, antibacterial, anti-inflammatory, antioxidant, antidiabetic, and antidepressant described for this plant (Boskabady *et al.*, 2011). Damask rose have also been made into jam, jelly and soup or are prepared as drinks like tea, primarily for their high Vitamin C content. It can be used in skin products and some makeup products. Rose water due to its specific flavor is used in desserts. They also have slightly medicinal uses (Niranjan *et al.*, 2015).

This study would confirm on the ability of each of the natural flower extract to show the presence of the parasite, clearly define its morphology and use them as alternative stain against Giemsa stain in parasitology laboratories.

## MATERIALS AND METHODS

**Plant collection:** Kujarat were collected from nearby market, Flowers of China rose, and Damask rose were collected from the university garden then it classified.

**Plant Extraction by distilled water:** Dried flowers of kujarat were washed with water to clean them from dust, left exposed to sun light for four consecutive days. 10 gm of the dried flowers were mixed with 100 ml distilled water and heated to 70 °C for three hours in a water bath then extracts were allowed to cool. Lastly, extracts undergone to purification technique.

**Alcoholic extraction:** Flowers of China rose and Damask rose were separated by hand picking, Fifty-gram of flower were mixed with 100 ml of 96% ethanol (0.5 g/ml). Then they were incubated at 4°C for 24-48 h for complete extraction of the stains. The extracts were then undergoing the purification technique.

**Purification of extract:** At the end of each extraction technique, the extracts were purified in to two steps filtration process. At first, the extract was filtered through wire mesh followed by filtration using Whatman filter paper. Filtrates were centrifuged at 5000 rpm for 30 min. The supernatant was collected into a reagent bottle and after proper labeling stored at 4°C until next usage.

**Leishmania strain:** *Leishmania donovani* strain was obtained from department of biology, Al-Mustansiriyah University. one drop from *L.*

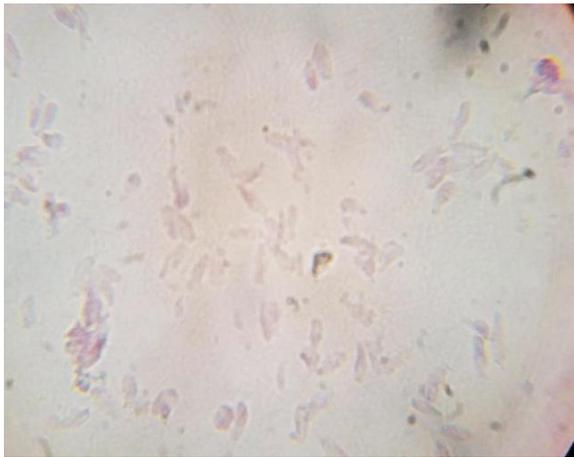
*donovani* culture on was put on clean dry glass slides thin smearing was made, left to dry then fixed with put one drop from absolute methanol and left to dry till staining.

**Staining of the parasite:** Five slides prepare for each flower. Slides put in different stains for 30 minutes and five slides colored with Giemsa stain in order to compare it with the pigments used in this study. Then washed with distilled water and left to dry, then all slides were examined under light microscope.

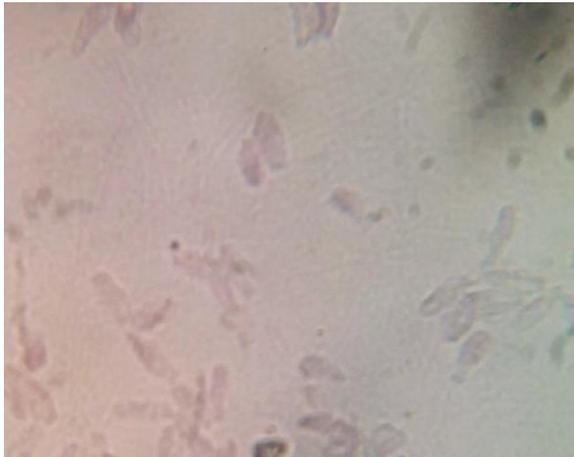
## RESULTS AND DISCUSSION

To determine the parasite for diagnosing purposes, three natural flower extracts were used as an alternative to conventional staining procedures. *L. donovani* promastigote responded to local natural flower dye extract and stained well in comparison with giemsa stain as control stain depending for parasite staining. The aqueous extract of *Hibiscus sabdariffa* flowers stained the parasite in pinkish color Fig 1, While alcoholic flowers extract of *Hibiscus rosasinensis* stained the parasite with bluish pink color Fig 2 and the alcoholic extract of *Rosa damascena* stained the parasite with pink red color Fig 3 The three-flower extract gave a good result when compared with giemsa staining Fig 4 which shows *Leishmania* promastigote staining with giemsa stain nuclei, cytoplasm and cell membrane. The result of the study may indicate that the natural stain can distribute through parasite's cell in different degree as Mohanad *et al.*, (2012) showed that when experimentally red beet extract had been used to stain different types of helminthes, all these stained helminthes tacked a good coloration with distinction their internal structure. Also, our result agrees with Okolie (2008) who stained the cyst of *Entamoeba coli* and *Entamoeba histolytica* with *Hibiscus sabdariffa* dye extract and the parasite cyst was seen as pinkish spherical bodies, this due to the presence of flavonoids pigments as Yoshikazu *et al.* (2009), who reported that the roses that stained Platyhelminthes imported pink to pink red color due to the presence of the predominant water-dissolvable anthocyanin stain. Previous studies proposed using watery extract of Kujarat as a natural stain because of the easy extraction of the kujarat flowers stain by boiling them in water and the other reason is the wide agriculture of these flowers in Iraq (Al-Sarraj, 1997: Al-Sarraj *et al.*, 1997). The natural fluid of kujarat has Nemours chemicals associated with its pigment, these chemicals may modify its power of reactivity as stains (Hashim, 2006). Using alcohol as extraction of China rose and damask flowers in this study were suggested

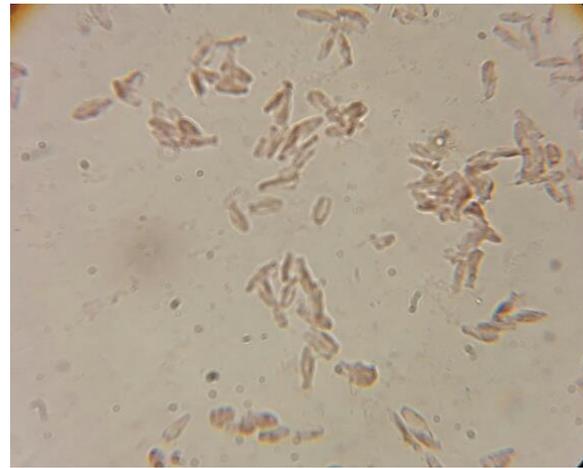
by Okolie (2006) who found that alcohol extraction produced good results because the dye stuffs were soluble in the ethanol used in the extraction Mohammed *et al.*, (2015) used the synthetic food coloring which were acidic substance because they have citric acid in their content for staining leishmania parasite and this kept their shape. So, the affinity of dyes for staining parasite is affected by solvent character, this agree with Avwioro (2011) who found that the tendency of dyes to stain tissue components was affected by factors as solvent characteristics and charge distribution of the dyes. Niranjana *et al.*, (2015) found that alcohol extract plant substances could be a good substitute for usual dyes.



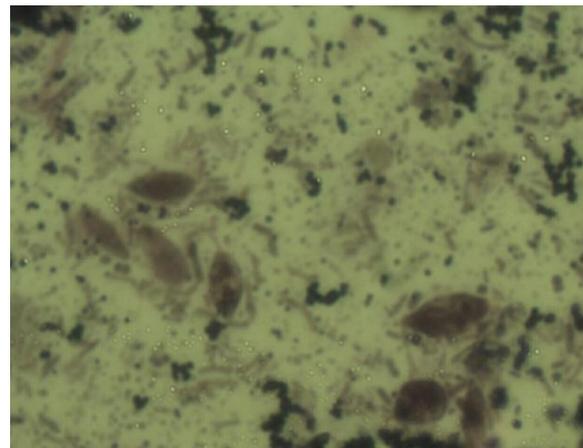
**Fig 1:** *L. donovani* promastigote stained by water extract of *Hibiscus sabdariffa* (2000X)



**Fig 2:** *L. donovani* promastigote stained by alcoholic extract of *Hibiscus rosa sinensis* (2000X)



**Fig 3:** *L. donovani* promastigote stained by alcoholic extract of *Rosa damascena* (2000X)



**Fig 4:** *L. donovani* promastigote stained with Geimsa stain (2000X)

The result of the study evidenced that the water extract of *Hibiscus sabdariffa* and alcoholic extract of China rose followed by Damask rose possess the potential to replace the conventional dye in staining procedure of *Leishmania* parasite in order to be used for diagnostic purposes in biology and medicine. It is recommended that more research be carried out on production of dried concentrates of these dyes in order to improve on their quality.

#### **Conclusion:**

The water extract of kujarat stained *L. donovani* promastigotes with pink color while the alcoholic extract of China rose stained *L. donovani* promastigotes with bluish pink color and the alcoholic extract of Damask rose stained *L. donovani* promastigote with red color. This result indicates the possibility of using natural flower dyes for diagnostic purposes in diagnostic laboratories of parasites.

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