A New Simplified Stain Extracted From The Petals of Kujarat Tea Flowers (Hibiscus Subdariffa)

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Summary

In the present study a new stain has been extracted for the first time from the potals of Kujarat tea flowers. Both watery and alcoholic extracts were prepared and examined for their ability to stain different human parasitic protozoa and helminths ova (in preserved and freshly passed stool samples).

The results of staining with Kujarat stain proved to be highly efficient when compared with routine stains (such as iodine and trichrome). In addition to that no differences were observed between the watery and alcoholic extracts, so the present work recommended the use of watery solution of stain (in saline) at the concentration of (8% wt./ vol.) for both routine and teaching purposes. The dried petals can be easily obtained from the local markets with a cheep price and then the stain can be easily prepared at any time.

Introduction

It is well known that different chemical stains are commonly used for the routine work (such as iodine, lishman's stain and others) to diagnose the parasitic protozoa and ova present in fecal smears (both in preserved and freshly passed stool samples) and also these which find in the blood (1-2)

According to Belding (3), stains which give clear results are the complicated and time consuming once, such as rapid iron hematoxylin method and others. Also the standerization of the stains is an important step as mentioned by Bahar et. al., and Winkind (4-5), it should be taken in consideration after testing procedure of a given stain. Recently Ali and A. Janabi (6) discovered a new natural stain, it extracted from the roots of the Wiled plant Fuwa (Rubia).

In the present study we have discoverd another new natural stain, it extracted from the petals of Kujarat tea flowers, the plant belongs to the Malvaceae family, species Hibiscus subdariffa . The plant grows in the middle and south of Iraq and in other tropical countries .

The purpose of this work is to find an alternative stain and test its efficient up-take by protozoan parasites or helminths ova present in the samples .

Material And Methods

Preparation of the stain = Different concentrations of the Kujarat stain was prepared (starting from 4% up to 10% wt./vol.) by boiling the dried petals directly in saline and alcohol for 5 mins. then the staining solution of each concentration was taken, boiled for another 10 mins., cooled and filtered. A few crystals of thymol was added to preserve the stain and to prevent contamination. The staining solution was used immediately (but improves when allowed to stand for 2-4 days at room tempreture, to encourage ripening) (7).

Staining of the samples :

Both freshly passed and preserved samples were examind by placing a small drop of stool sample on a clean slide then two drops of stain was added, mixed by a wooden sticks, coversliped and examined under the high power of the light microscope.

The results of the stain uptake by the parastic protozoa and ova were compared with the iodine stain.

Properties of the staining solution :

The staining solutions for both watery and alcoholic extracts were dark red-dark violet in colour, and acidic in nature. The stain was stable at room tempreture for one-two weeks and many months at 4C°.

Results

In the present work, the detailed results of staining for protazon parasites and helminths ova are shown in tables 1,2 and 3. Both extracts (with the concentration of 8% wt./vol., in saline) gave same results. The stain was provide to be highly efficient to stain different structures of the organisms.

Discussion :

The overall results of this study indicate that the watery and alcoholic extracts of Kujarat stain gave typical results in staining different structures of trophozoite, cysts and ova present in testing samples, which means that extract is capable easily to penetrate inside the organisms and colouring the internal structures without causing any damage. Our results were good and clear as the results obtained by Ali and Al-Janabi(6) but in a different colour.

The stain can be easily prepared from the petals of the plant by bioling the petals directly in saline and in alcohol.

From the results obtained in this work we can conclud the followings:

1- The Kujarat stain can be used as an alternative stain for parasitic protozoan and helminths ova for routine and teaching purposes because of it is highly efficient stain and economic.

2- The new present stain can be

considerad as a natural one step rapid stain.

3- There is no significant differences between the watery and alcoholic exracts of the stain.

4- The best concentration of the stain is 8% (wt./vol.) in saline. The work is now in progress to use this stain for otherpurposes.

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Table 1 : Parasitic protozoa both in preserved and freshly passed samples (amoeba) .

Structures	Iodine stain	Kujarat stain (tested stain)
E histolytica trophozoit		
Cell wall	black yellow	cler red
Cytoplasm	pale yellow	light pinkish
Nucleus	brown	pinkish
Karyosome	brown	bright pink
Food vacules	brith green	pinkish
E. hsitolytica cytst		
Cyst wall	yelowish green	clear red
Cytoplasme	yellow	light binkish
Nucleus	dark brown	pinkish
Karyosome	brown	bright dark pink
Chromatodal baars	mahogory	pale pink
Glycogen mass	dark brown	
E. trophozoite		pale pink
Cytoplasme	pale yellow	pinkish
Nucleus	brown	pinkish
Chromatodal baars	brown	
Food vacules	yellowish	
E. coli syst		clear red
Cyst wall	yellow	light pinkish
Cytoplasme	pale yellow	pinkish
Nucleus	brown	bright
Karyosome	dark brown	pink
Chromatodal bodies	dark brown	
Endolimax nana cysts		clear dark red
Cyst wall	pale green	light pinkish
Cytoplasme	light brown	pinkish
Nucleus	brown	bright dark
Karyosome	dark brown	pink
Idomoeba butschlii cyst		
Cyst wall	pale green	clear dark red
Cytoplasme	light brown	light pinkish
Nucleus	brown	pinkish
Karyosome	dark brown	bright dark pink
	dark brown	pale pink

Table 2 : Parasitic flagellates

Giardia lamblia		
Trophozoit and cyst		
wall	dark brown	cler red
Cytoplasm	pale yellow	light pinkish
Nucleus	brown	bright pink
Karyosome	dark brown	bright dark pink
Axostyle	black	light binkish
Parbasal bodies	brown	light binkish
Chilomastix mesnili cyst		
Cell wall	faint green	clear red
Cytoplasme	pale green	pale pinkish
Nucleus	bright green	bright pink
Leishmania spp. from		
culture		clear red
(permenant slides)		pale pinkish
Cell wall		bright blue pinkish
Cytoplasme		
Nucleus		

Table 3 : Parasitic helminth ova in fecal sample

Structures	Iodine stain	Kujarat stain (tested stain)
Hymenolepis nana		
egg shell	yellowish brown	pinckish red
embryophore	pale brown	pale pink
polar filaments	pinkish	pink
H. diminuta		
egg shell	yellowish brown	pinckish red
embryophore	pale brown	pale pink
polar filaments	pinkish	pink
Ascaris lumbricoides		
Albuminous layer	brown	pinckish red
embryo	pale brown	pink
Hook worms		
egg shell	dark brown	clear red
embryo	yellowish brown	pinkish
Taenia species		
straited layer	black	clear red
embryo hooklets	black	red

صبغة جديدة وبسيطة كمستخلصة من تويج زهرة شاي كجرات

الخلاصة

في الدراسة الحالية تم ولاول مرة تحضير صبغة جديدة مستخلصة من التويجة لزهرة نبات شاي كجرات .

اثبتت النتائج ان الصبغة لها كفاءة عالية عند مقارنتها مع الصبغات الروتينية . كما نلاحظ اي فروقات جوهرية في النتائج بين المستخلص الكحولي او المائي .

تم تحضير الصبغة من المستخلص المائي او الكحولي للاوراق المذكورة اعلاه واستخدمت في صبغ الاطوار النشطة والمتكيسة لمعظم الاوالي المتطفلة في الانسان وكذلك في صبغ بيوض معظم الديدان الشائعة (الديدان الشريطية ، الديدان الدبوسية ، الديدان الشصية وغيرها) لبراز الانسان المحفوظ او النماذج الطرية .

اعطى تركيز (٨٪ وزن/حجم) افضل النتائج ، لذا توصي الدراسة الحالية باستخدام المستخلص المائي وبتركيز (٨٪) كبديل للصبغات الروتينية (صبغة اليود) او الكروم الثلاثي) لاغراض التحري المختبري عن الطفيليات وللاغراض التعليمية ، اضافة الى سهولة الحصول الحصول على زهور النبات (بشكل مجفف) من الاسواق المحلية وبسعر رخيص ، وبالتالي سهولة الحصول على هذه الصبغة الطبيعية باستمرار .