Original Article

Screening Of Natural Stains From Indian Plants In Rat Skin Tissue For Histological Applications

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Abstract

Histopathological diagnosis involves examination of slides stained by chemical stains which are health hazard to researchers, technicians and pathologists. This study screened plant based natural stains for histological applications. About 176 test staining solutions were prepared from 11 plant extracts and the staining ability in rat skin tissue was compared with the routinely used Hematoxylin and Eosin (H&E) stain. Punica granulatum, Bixa orellana, Termanalia chebula, Rheum emodi, Rubia tinctorium, Beta vulgaris, Lawsonia inermis, and Tagetes erecta extracts showed more staining intensity. This is the first study to demonstrate the staining ability of Termanalia chebula, Rheum emodi, and Tagetes erecta in biological tissue. Among all plants, Rubia tinctorium and Beta vulgaris showed better staining ability, but lower than H&E. By modifying the concentration, pH, and staining time, we could get better natural stains for histology.

Keywords - Natural stains, Hematoxylin, Eosin, plant based stains, skin tissue

1. INTRODUCTION

The study of biological tissues to appreciate the diseased cells using a microscope is called histopathology. Different processes are involved in converting un-stained tissues to stained tissue sections which include fixation, dehydration, embedding, sectioning and staining (Ramamoorthy et al., 2016). Cellular differentiation of tissues has become more accurate with the use of specific stains, which is due to increase in optical differentiation of cellular elements either by alteration of contrast or impartation of colour (Ajileye et al., 2015). Routinely histopathology slides stained with Hematoxylin and Eosin (H&E) are used. Hematoxylin, which is a basic dye stains the acidic structures like nucleus and eosin, being an acidic dye stains the basic structures like cytoplasm, connective tissue etc (Lahiani et al., 2018). Apart from this, certain tissue specific special stains are also used. Hence, the staining materials in histology is very useful in clinical diagnosis and research in medical field. (Bordoloi et al., 2017).

Hematoxylin is a natural dye obtained from the heartwood of logwood Mexican tree Haematoxylon campechianum (Ajileye et al., 2015). Easy differentiation, durability and comparative permanency are the advantages of hematoxylin but there is an increase in cost for many years particularly in countries where it is imported (Benard, 2008, Mohandas et al., 2019). Eosin, a very efficient stain is a synthetic Xanthene dye but it is hazardous to animal and human health (Mohandas et al., 2019). The continuous exposure of chemicals from synthetic stains can affect the health of pathologists, technicians etc. The other disadvantages of synthetic stains like expensiveness of cost, non-biodegradability also made natural stains gaining importance to substitute the synthetic stains (Sudhakaran et al., 2018)

Many natural dyes and stains are obtained from different parts of the plants which are non-poisonous, relatively less health hazardous, non-carcinogenic, less polluting and less toxic (Siva, 2007). These natural dyes are used in many industries like textiles, paper, food industries, leather, paint etc (Verma and Gupta, 2017). The use of natural stains from very few plant extracts is explored in many studies as a substitute to H & E staining, but there are many more coloring plants yet to be reported. In this study, we screened six new plants such as Acacia nilotica, Areca catechu, Indigofera tinctoria Termanalia chebula, Rheum emodi and Tagetes erecta which were not reported earlier for its staining property in biological tissues. We also selected few reported plants with new solvents to check whether we could get better staining ability. The aim of this study was to screen plant based natural stains for histological applications in rat skin tissue.

2. MATERIALS AND METHODS

2. 1. Rat skin tissue sections:

The Institutional animal ethical clearance was obtained to collect the skin tissues for the study (Approval no: IAEC/KMC/10/2020). After euthanasia, the skin tissues were collected from rats and kept in 10% formalin for fixation. The tissue blocks were prepared by standard tissue processing procedures like dehydration with different grades of alcohol, clearing in xylene and wax infiltration with paraffin wax. Skin tissues were sectioned at 4 µm thickness. A total of 1,770 tissue sections have been prepared for the study and five tissue sections were stained with each staining solution.

2.2. Plant extracts and different staining solutions:

A total of 11 plant based natural extracts were purchased from Skymorn Herbs and Dyes Exports, Ghaziabad, Uttar Pradesh as well as Vital Herbs, Delhi. From each extract, 16 different staining solutions were prepared by mixing the extracts of low and high concentrations in different solvents like distilled water, ethanol, 3% acetic acid and ethyl acetate. Alum was used as mordant in half of the staining solutions. Totally, 176 test staining solutions (11 extracts X 16 different solutions) were prepared and screened for staining property in rat skin tissue. H&E stain was used as standard staining solution. **(Table 1).**

Table 1: Preparation of 16 different staining protocol from an extract

S.No.	Extract	Dissolving solvent (5 ml)	Mordant (0.5 gm)	Staining time
	Extract low concentration			
1	0.2 gm / 5 ml	Distilled water	-	30 minutes
2	0.2 gm / 5 ml	Ethanol	-	30 minutes
3	0.2 gm / 5 ml	3% Acetic acid	-	30 minutes
4	0.2 gm / 5 ml	Ethyl acetate	-	30 minutes
5	0.2 gm / 5 ml	Distilled water	Alum	30 minutes
6	0.2 gm / 5 ml	Ethanol	Alum	30 minutes

7	0.2 gm / 5 ml	3% Acetic acid	Alum	30 minutes
8	0.2 gm / 5 ml	Ethyl acetate	Alum	30 minutes
	Extract high concentration			
9	0.4 gm / 5 ml	Distilled water	-	30 minutes
10	0.4 gm / 5 ml	Ethanol	-	30 minutes
11	0.4 gm / 5 ml	3% Acetic acid	-	30 minutes
12	0.4 gm / 5 ml	Ethyl acetate	-	30 minutes
13	0.4 gm / 5 ml	Distilled water	Alum	30 minutes
14	0.4 gm / 5 ml	Ethanol	Alum	30 minutes
15	0.4 gm / 5 ml	3% Acetic acid	Alum	30 minutes
16	0.4 gm / 5 ml	Ethyl acetate	Alum	30 minutes

2.3. Tissue staining experiments:

In order to study the staining ability of plant extracts in rat skin tissue, we conducted two sets of experiments. Experiment 1 was aimed to study whether the plant based stains could replace the Eosin and experiment 2 was aimed to study whether the plant based stains could replace Hematoxylin.

2.3.1 Comparing natural stains with eosin

In experiment 1, hematoxylin and eosin served as standard staining solution, whereas in the test solutions, the eosin was replaced with 176 plant based test staining solutions, which were prepared from 11 plant extracts as mentioned above. Here, we had 177 solutions and 5 skin tissue sections were used for each solution, thus a total of 885 skin tissue sections were used for the experiment 1. **(Table – 2)**

Table 2: Staining protocol for comparing natural stains with eosin

	Hematoxylin and Eosin		**Hematoxylin and natural stain**	
No	**Staining solution**	**Time**	**Staining solution**	**Time**
1	Xylene I	5 minutes	Xylene I	5 minutes
2	Xylene II	5 minutes	Xylene II	5 minutes
3	Xylene III	10 minutes	Xylene III	10 minutes
4	100% Alcohol	2 minutes	100% Alcohol	2 minutes
5	70% Alcohol	2 minutes	70% Alcohol	2 minutes
6	50% Alcohol	2 minutes	50% Alcohol	2 minutes
7	Running water	2 minutes	Running water	2 minutes
8	Hematoxylin	2 minutes	Hematoxylin	2 minutes
9	Running water	1 minutes	Running water	1 minutes
10	Acid alcohol	2 dips	Acid alcohol	2 dips
11	Running water	10 minutes	Running water	10 minutes
12	**Eosin**	**1 minute**	**Natural stain**	**30 minutes**
13	100% alcohol	1 dip	100% alcohol	1 dip
14	70% alcohol	2 minutes	70% alcohol	2 minutes
15	Xylene	5 minutes	Xylene	5 minutes

2.3.2. Comparing natural stains with hematoxylin

In experiment 2, the Hematoxylin was replaced with 176 plant based test staining solutions, which were prepared from 11 plant extracts as mentioned above. While counterstaining the plant based test staining solution with eosin, eosin completely masked /overwhelmed it, hence the actual staining ability of plant based test staining solutions could not be assessed. So, in this experiment, we used only plant based natural test staining solution by skipping eosin. To match this, the slides in standard group were stained with only hematoxylin by skipping eosin. Here, we had 177 solutions and 5 skin tissue sections were used for each solution, thus a total of 885 skin tissue sections were used for the experiment 1 **(Table – 3)**

Table 3: Staining protocol for comparing natural stains with hematoxylin

	Hematoxylin only		**Natural stain only**	
No	**Staining solution**	**Time**	**Staining solution**	**Time**
1	Xylene I	5 minutes	Xylene I	5 minutes
2	Xylene II	5 minutes	Xylene II	5 minutes
3	Xylene III	10 minutes	Xylene III	10 minutes
4	100% Alcohol	2 minutes	100% Alcohol	2 minutes
5	70% Alcohol	2 minutes	70% Alcohol	2 minutes
6	50% Alcohol	2 minutes	50% Alcohol	2 minutes
7	Running water	2 minutes	Running water	2 minutes
8	**Hematoxylin**	**2 minutes**	**Natural stain**	**30 minutes**
9	Running water	1 minutes	Running water	1 minute
10	Acid alcohol	2 dips	Acid alcohol	2 dips
11	Running water	10 minutes	Running water	10 minutes
12	100% alcohol	1 dip	100% alcohol	1 dip
13	70% alcohol	2 minutes	70% alcohol	2 minutes
14	Xylene	5 minutes	Xylene	5 minutes

2.4. Grading of staining:

All the stained tissue sections in slides were observed under light microscope (Labomed Lx500) and the images was taken with MiaCam CMOS AR 6pro microscope camera connected to image AR pro software. All the stained tissue sections were observed by three independent blinded observers (pathologists) for its staining ability and grades (poor, good, excellent) were given depending on the staining ability as follows;

2.4.1. Comparing natural stains with eosin

Grade 1 (poor) - if there is no clear morphology of tissue structures and no contrast seen between the stains; **Grade 2 (good)** - if the morphology of the tissue structures is clearly seen but no contrast seen between the stains; **Grade 3 (excellent) -** if the morphology of tissue structures clearly seen and good contrast seen between the stain.

2.4.2. Comparing natural stains with hematoxylin

Grade 1 (poor)- If the staining solutions stains all the tissue structures without any specificity or If the staining solution could not attach to the tissue structure. **Grade 2 (good) -** If the staining solution stains darker in acidic structures compared with the basic structures

Grade 3 (excellent)- If the staining solution specifically stains only the acidic structures

2.5. Statistical analysis:

The data was expressed in terms of frequency percentage of good/excellent/poor. The ability to substitute the eosin/haematoxylin with natural stain were tabulated and compared.

3. RESULTS

The frequency percentage of grades depending on the staining ability of plant extracts are tabulated. All the tissue sections stained with H & E staining were in grade 3 (excellent). None of the plant extracts used in this experiment showed Grade 3, but many showed grade 2.

3.1. Comparing natural stains with eosin

Among 11 plants and their 16 staining solutions, 8 staining solutions have shown better staining ability (Grade 2, Good). They are Punica granulatum (0.4 gm / 5 ml distilled water with alum), Bixa orellana (0.4 gm / 5 ml distilled water), Termanalia chebula (0.4 gm / 5 ml distilled water), Rheum emodi (0.4 gm / 5 ml alcohol), Rubia tinctorium (0.4 gm / 5 ml distilled water), Beta vulgaris (0.4 gm / 5 ml alcohol), Lawsonia inermis (0.4 gm / 5 ml alcohol), and Tagetes erecta (0.4 gm / 5 ml Ethyl acetate). Extracts with high concentration showed more staining intensity than low concentration. When comparing with all the plants, Rubia tinctorium (0.4 gm / 5 ml distilled water) and Beta vulgaris (0.4 gm / 5 ml 3% Acetic acid) showed better stained ability **(Table 4, Fig.1-2).**

Table 4: **Frequency Percentage Staining ability of different extracts on skin tissue as a substitute to eosin**

Plant extracts / standard	**Different staining solutions prepared as per table 1**															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Haematoxylin & Eosin*	**E-100***															
Punica granulatum*	G-80 P-20	NS	NS	NS	G-60 P-40	NS	NS	NS	G-60 P-40	NS	NS	NS	**G-100***	NS	NS	NS
Bixa orellana*	G-60 P-40	P-100	P-100	P-100	G-40 P-60	NS	P-100	NS	**G-100***	P-100	P-100	P-100	G-80 P-20	NS	P-100	NS
Termanalia chebula*	G-60 P-40	G-60 P-40	P-100	G-60 P-40	P-100	NS	P-100	NS	**G-80*** **P-20**	G-60 P-40	P-100	P-100	P-60 G-40	NS	P-100	NS
Rheum emodi*	P-60 G-40	G-80 P-20	G-80 P-20	NS	P-80 G-20	G-100	P-100	NS	G-60 P-40	**G-100***	G-60 P-40	NS	G-60 P-40	G-80 P-20	P-100	NS
Acacia nilotica	P-80 G-20	NS	P-100	NS	P-60 G-40	NS	P-100	NS	P-80 G-20	NS	P-100	NS	P-100	NS	P-100	NS
Rubia tinctorium*	G-80 P-20	P-100	P-100	P-100	G-60	NS	P-100	NS	**G-100***	P-80 G-20	P-100	NS	G-60 P-40	NS	P-100	NS
Indigofera tinctoria	P-100	NS	NS	NS	P-100	NS	NS	NS	P-100	NS	NS	NS	NS	P-100	NS	NS
Areca catechu	P-80 G-20	NS	G-60 P-40	NS	G-60 P-40	NS	G-60 P-40	NS	G-60 P-40	NS	G-60 P-40	NS	G-60 P-40	NS	P-80 G-20	NS
Beta vulgaris*	G-60 P-40	G-80 P-20	G-80 P-20	NS	P-80 G-20	G-80 P-20	P-100	NS	G-60 P-40	**G-100***	P-60 G-40	NS	G-60 P-40	G-80 P-20	P-100	NS
Lawsonia inermis*	G-60 P-40	G-60 P-40	P-80 G-20	NS	P-60 G-40	G-80 P-20	P-100	NS	G-60 P-40	**G-100***	P-60 G-40	NS	G-60 P-40	G-60 P-40	P-100	NS
Tagetes erecta*	NS	P-60 G-40	NS	G-80 P-20	NS	NS	NS	NS	NS	G-80 P-20	NS	**G-80*** **P-20**	NS	NS	NS	NS

E- excellent (Grade 3), G- good (Grade 2), P- poor (Grade 1), NS- Not soluble, values are expressed in %, * indicates best staining solutions

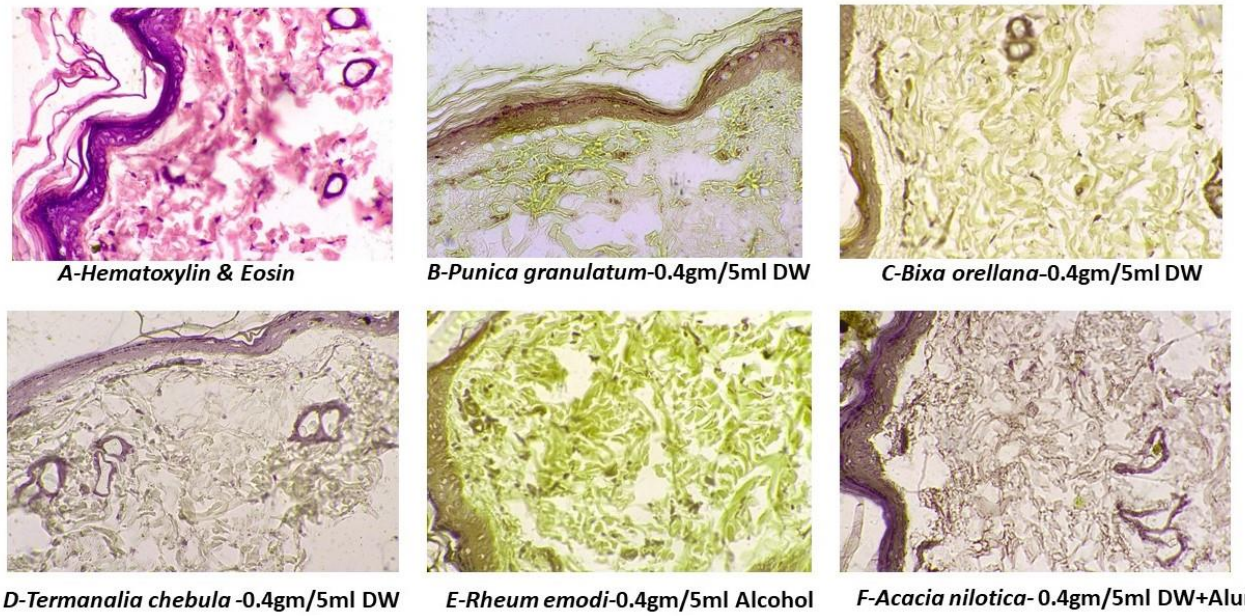

Fig 1- Staining ability of different extracts on skin tissue as a substitute to eosin (A-F) (100x)

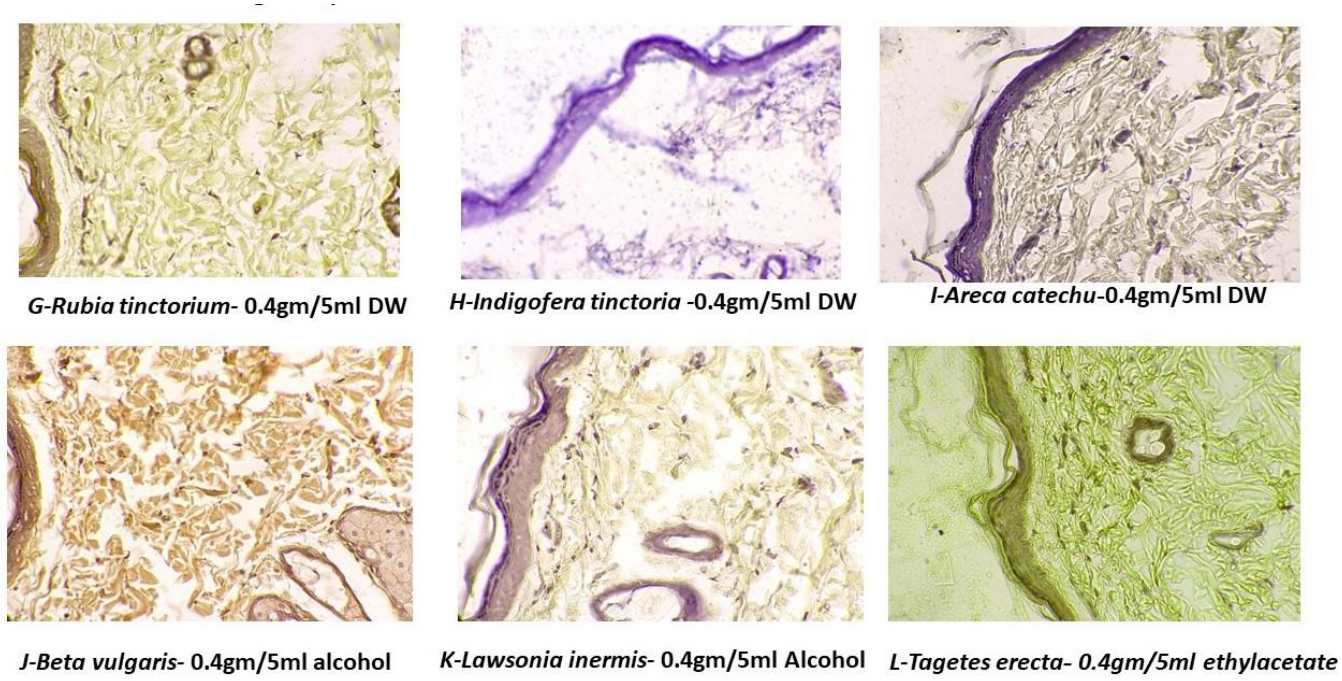

Fig 2- Staining ability of different extracts on skin tissue as a substitute to eosin (G-L) (100x)

3.2. Comparing natural stains with hematoxylin

Among 11 plants and their 16 staining solutions, only 2 staining solutions have shown better staining ability (Grade 2, Good), which are Rubia tinctorium (0.4 gm / 5 ml distilled water with alum), and Beta vulgaris (0.4 gm / 5 ml 3% Acetic acid with alum). Extracts with high concentration with the addition of alum showed more staining intensity than low concentration and without alum. **(Table 5, Fig.3-4)**.

Table 5: **Frequency Percentage Staining ability of different extracts on skin tissue as a substitute to hematoxylin**

Plant extracts / standard	**Different staining solutions prepared as per table 1**															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Haematoxylin*	**E-100***															
Punica granulatum	P-100	NS	NS	NS	P-100	NS	NS	NS	P-100	NS	NS	NS	P-100	NS	NS	NS
Bixa orellana	P-100	P-100	P-100	P-100	P-100	NS	P-100	NS	P-100	P-100	P-100	P-100	P-100	NS	P-100	NS
Termanalia chebula	P-100	P-100	P-100	P-100	P-100	NS	P-100	NS	P-100	P-100	P-100	P-100	P-100	NS	P-100	NS
Rheum emodi	P-100	P-100	P-100	NS	P-100	P-100	P-100	NS	P-100	P-100	P-100	NS	P-100	P-100	P-100	NS
Acacia nilotica	P-100	NS	P-100	NS	P-100	NS	P-100	NS	P-100	NS	P-100	NS	P-100	NS	P-100	NS
Rubia tinctorium*	P-100	P-100	P-100	P-100	P-100	NS	P-100	NS	P-100	P-100	P-100	NS	**G-100***	NS	P-100	NS
Indigofera tinctoria	P-100	NS	NS	NS	P-100	NS	NS	NS	P-100	NS	NS	NS	NS	P-100	NS	NS
Areca catechu	P-100	NS	P-100	NS	P-100	NS	P-100	NS	P-100	NS	P-100	NS	P-100	NS	P-100	NS
Beta vulgaris*	P-100	P-100	P-100	NS	P-100	P-100	P-100	NS	P-100	P-100	P-100	NS	G-80 P-20	P-100	**G-100***	NS
Lawsonia inermis	P-100	P-100	P-100	NS	P-100	P-100	P-100	NS	P-100	P-100	P-100	NS	P-100	P-100	P-100	NS
Tagetes erecta	NS	P-100	NS	P-100	NS	NS	NS	NS	NS	P-100	NS	P-100	NS	NS	NS	NS

E- excellent, G- good, P- poor, NS- Not soluble, values are expressed in %, * indicates best staining solutions

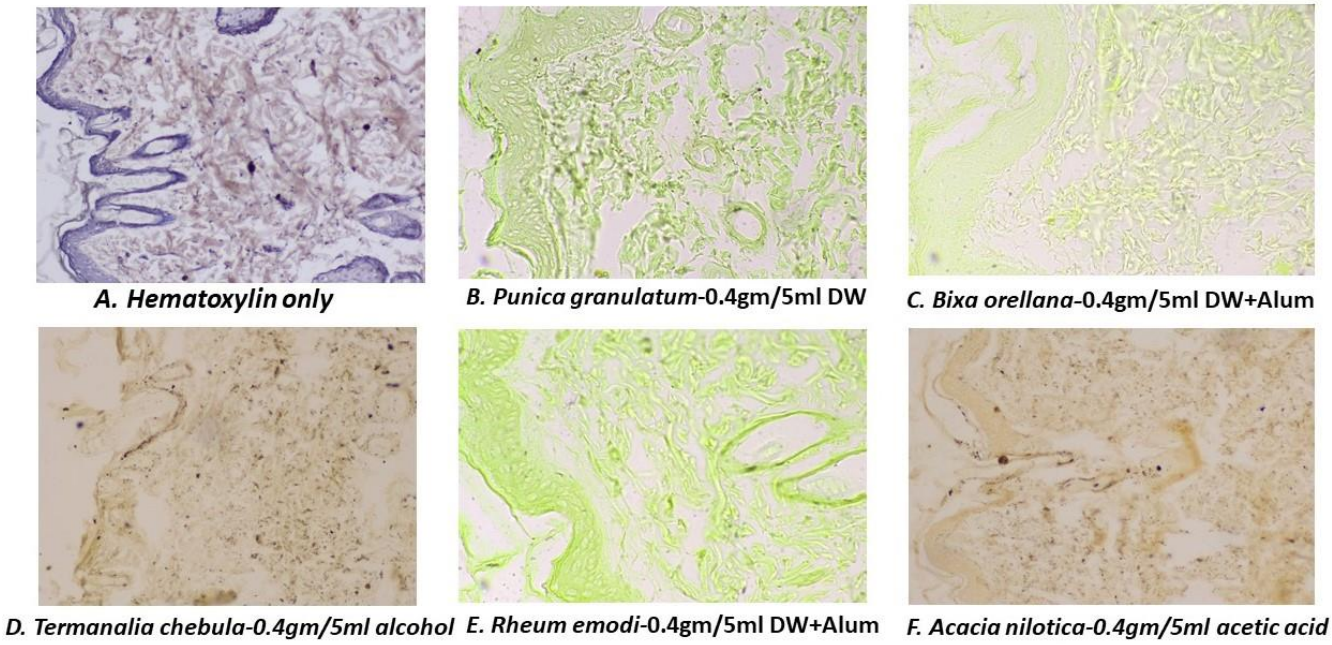

Fig 3- Staining ability of different extracts on skin tissue as a substitute to Hematoxylin (A-F) (100x)

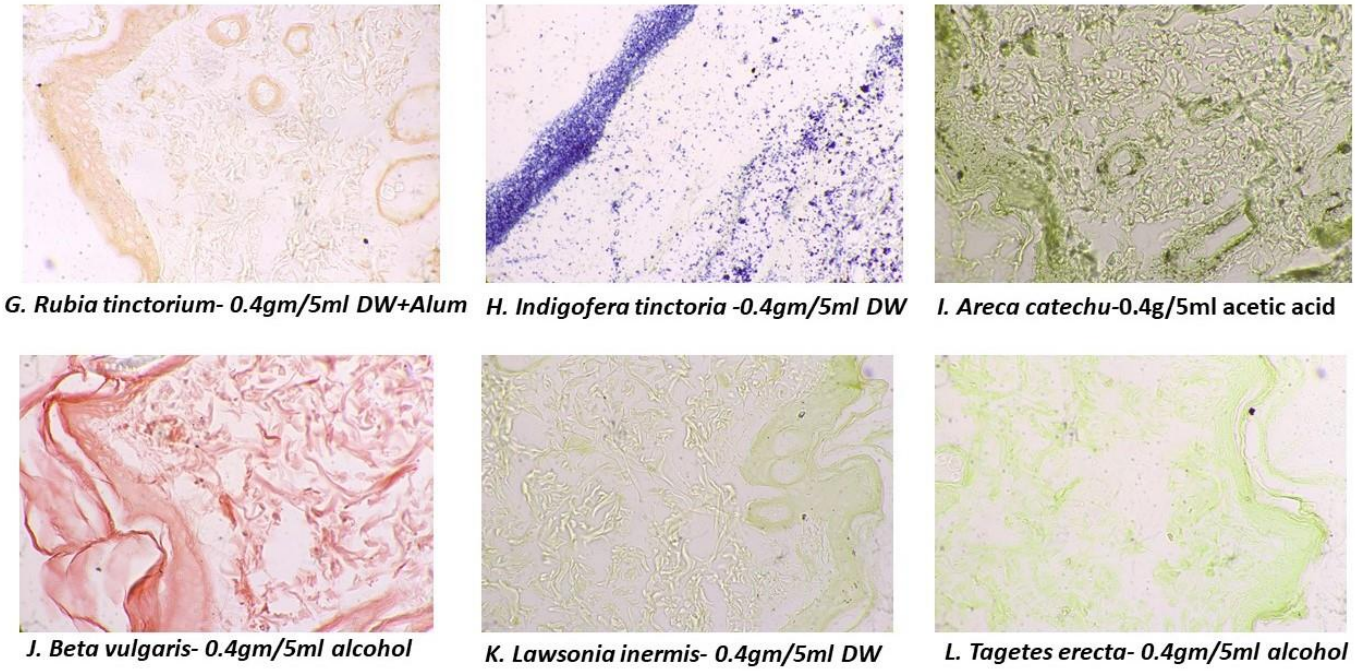

Fig 4- Staining ability of different extracts on skin tissue as a substitute to Hematoxylin (G-L) (100x)

DISCUSSION

Histological staining involves a series of technical processes in tissue sample preparation including staining with histological stains for microscopic studies (Alturkistani et al., 2015). H & E staining is the basic routine staining used in histopathological diagnosis. A normal H & E staining shows blue colour in cell nuclei due to hematoxylin and varying degrees of pink colour in cell cytoplasm and connective tissues due to eosin (Lahiani et al., 2018). Natural plant products containing different dye compounds have been used for dyeing in textile, food, cosmetic industries. These industries use mordants to stain which act as a bridge between the material which is dyed and the stains (Avwioro, 2011).

Awareness on the health and environmental hazards of synthetic dyes has led to the research on natural stains as histological stains. In this study, we have attempted to screen many plant extracts which has been already used in dye industry to be used as a substitute to hematoxylin or eosin by preparing different staining solutions with distilled water, alcohol, 3% acetic acid and ethyl acetate to utilise their varying chemical properties. Potash alum which is a cheap and safe mordant was also added to analyse its staining efficiency.

Eosin is an acidic or anionic dye with a negative charge which react with the cationic groups of acidophilic structures like proteins and cytoplasm and is referred as cytoplasmic stain (Veuthey et al., 2014). Most of the commercial preparations of eosin stains use either Eosin B or Eosin Y in the solvent alcohol which produces three tone effect showing three different shades between collagen, blood vessels and smooth muscles (Feldman and Wolfe, 2014). Our experimental study on 11 natural extracts to analyse the capability to substitute eosin has revealed that while few of the extracts with different staining solutions showed good intensity of staining, the contrast between the acidic and basic structures was not comparable with H & E staining. Even though few studies have been done on Punica granulatum, Bixa orella, Rubia tinctorum, Beta vulgaris, Lawsonia inermis, this study has used different staining solutions which was different from others.

The alkaloid form of granatonine, (N methyl granatonine) is responsible for colouring in Punica granulatum (pomegranate peel) (Kulkarni et al., 2011). Earlier report by Kuskulu et al showed light staining when the Pomegranate extract was used without mordant (Kusculu and Aslan, 2019; Kusculuo, 2018). In our study, solution prepared by mixing Pomegranate extract in distilled water with alum gave more intensity of staining. Thus, pomegranate extract with mordant could be studied further.

The main dyeing compound present in Bixa orella (Annato) is Bixin which is a red carotenoid pigment (Vilar et al., 2014). Previous study by Nnaemeka et al, showed that the Annato could not stain the tissue better than eosin (Nnaemeka Okarie et al., 2019). In our study also, distilled water solvent has shown better staining compared with other staining solutions, but not better than eosin.

Rubia tinctorum is a source of anthroquinine compound containing natural dye pigment Alizarin (Angelini et al., 1997; Golcu et al., 2009). Alizarin is a special stain for calcium which stains the calcioreceptive or calcifying zone of the collagenous matrix where calcium salts are deposited with its wide application in staining calcium casts in the kidney, pathological calcification in hypervitaminosis and artificial deposits of calcium (Schorr et al., 1959). In our study, Rubia tinctorum (Madder) extract with distilled water has shown good staining property in skin tissue.

Beta vulgaris (beetroot) shows abundant of betalein pigment with its variant betacyanin and betaxanthins giving red and yellow pigment respectively (Arthikha and Madhanasundareswari, 2019). The well-known colouring properties of Lawsonia inermis (Henna) is due to the presence of Lawsone, a red orange pigment (Bhuiyan et al., 2017). Few earlier studies with henna has inferred henna as a possible substitute to eosin (Adisa et al., 2017; Raju et al., 2018). Alcohol based staining solutions of Beta vulgaris and Lawsonia inermis showed slightly increased staining intensity compared with other staining solutions. However, our experiment showed that the staining ability of henna was inferior to eosin.

In our study, we screened six new plants for staining ability in biological tissues. Among them,

Acacia nilotica, Areca catechu and Indigofera tinctoria did not stain the skin tissue, whereas Termanalia chebula, Rheum emodi (Himalayan Rhuharb), and Tagetes erecta (marigold) plants stained the skin tissue. This is the first study to demonstrate the staining ability of Termanalia chebula, Rheum emodi, Tagetes erecta in biological tissue. Termanalia chebula extract contains tannin, when mixed with distilled water showed better staining ability when compared with other staining solutions. The Rheum emodi stain gave a greenish yellow staining with slightly more intensity when dissolved in alcohol and alum. Tagetes erecta is a rich source of lutein which is a carotenoid pigment.(Vastrad et al., 2017) Ethyl acetate based staining solutions prepared from Tagetes erecta showed slightly increased staining intensity compared with other staining solutions.

According to Kabir et al work on dyeing of fibres which might be applicable for tissue staining, a weak co-ordination complex might be formed by alum which will block the dye and reduce the interaction between the dye and tissue (Kabir et al., 2020). Studies on dyeing fabrics with extracts like Punica granulatum, Bixa orella, Termanalia chebula, Rheum emodi, Tagetus erecta has inferred that iron mordant has increased colour strength and colour fastness compared with other mordants (Jamadar and Sannapapamma, 2018; Nayak, 2014; Shabbir et al., 2016; Tutak et al., 2014; Zaman et al., 2018). Higher colour strength of ferrous sulphate compared with other

mordants might be due to the action of ferrous sulphate as transition metal ions forming many complexes with the dyeing molecules (Kabir et al., 2020). In this study we have used only alum as mordant because of its easy availability and cheaper price. Further studies can be concentrated on using ferrous sulphate as mordant.

Haematoxylin is a popular basic or cationic dye having a positive charge reacting with the anionic groups like phosphates, sulphates and carboxylates. This dye binds to the phosphate group of basophilic components like nucleic acids in DNA and RNA of nucleus and is referred to as nuclear stain (Veuthey et al., 2014). Haematoxylin cannot stain tissues unless a mordant is added. This mordant which is a metallic salt forms a link between the tissue and dye called 'lake' which is positively charged and acts as a cationic dye (Veuthey et al., 2014). It gives blue colour when potash alum is used and black colour when iron alum is used (Avwioro, 2011). In our second experiment, which was done to evaluate the efficiency of the 11 extracts as a substitute for haematoxylin, the haematoxylin in the standard H & E procedure was replaced by the natural staining solutions. Eosin was skipped in the procedure, so that staining of hematoxylin specifically to acidic structures can be visualised, thus preventing eosin from masking the colour of natural stain due to its dark pink colour.

Most of the staining solutions stained all the tissue structures without any specificity to acidic structures.

In our study, few staining solutions prepared from by Rubia tinctorium, and Beta vulgaris showed slightly darker stain to acidic structures comparatively to basic structures. A study on Beta vulgaris by Udonkang et al has stained basic histological structures like keratin, muscle etc (Udonkang et al., 2018). Whereas a study by Finbarrs-Bello has shown a hematoxylin like effect staining acidic structures like nucleus (Finbarrs- Bello et al., 2019). Beta vulgaris, Rubia tinctorium have the capacity to be used as an acidic and basic stain with its chemical modifications. Further studies could be done with these staining solutions by using a lighter, more specific counterstain which would stain basic structures or by modifying the staining solutions by increasing the concentration, changing the pH etc.

In this study, we used 11 plant extracts with their 16 different types of staining solutions for the screening. We used two concentrations (0.2 g, 0.4 g in 5ml) of extracts, different solvents (distilled water, ethanol, acetic acid, ethyl acetate) and alum as a sole mordant with the staining time of 30 minutes. Extracts at 0.4 mg in 5 ml showed better staining intensity. However, future studies could be conducted using higher concentration of extracts and mordants other than alum. Beta vulgaris and Rubia tinctorium could be studied in detail after some modifications to make it more basic nature, that could be comparable with hematoxylin.

CONCLUSION

The rising trend of eco-friendly nature has led to an urge to replace chemical synthetic dyes with natural dye in all industries. Even though none of the stains prepared from the eleven plants gave comparable results to hematoxylin and eosin, most of the stains has good staining ability when compared with eosin. Beta vulgaris and Rubia tinctorium has the ability to substitute hematoxylin after some modifications to make it more basic nature. When staining conditions like staining time, temperature, pH is modified with addition of mordant many natural extracts can produce a darker stain with better staining ability in tissues.

Conflict of interest: The authors declare that they have no conflicts of interest

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Ethical approval: This study is approved by the Institutional animal ethics committee Approval no: IAEC/KMC/10/2020.

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