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Review Article

Utilization of Natural Dyes Substances for Histological Staining: A Review

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A B S T R A C T

Histological staining is one the technical processes in the preparation of tissue samples by coloring using histological staining to assist in the study. Natural dyes as an alternative solution to replace synthetic dyes because it can affect human health such as skin allergies, digestive system and respiratory system. This review article aims to determine the quality of natural dyes used to color tissue in the process of making animal and plant tissue preparations. Some of the plants used as natural dyes, coloring indicates a change in tissue preparations. From some plants used for natural dyes in the histology of animal and plant tissues, chemical compounds contained there in that can provide colors such as flavonoids, alkaloids, saponins, tannins, anthocyanins, betasianins, and curcuminoids. The resulting dye looked good or not is influenced by the pH, concentration, staining time, method and solvent used in the staining.

Keywords: Natural dyes, Histology, Tissue.

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INTRODUCTION

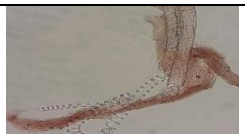
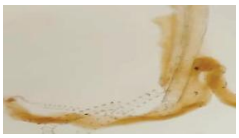
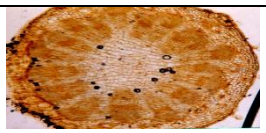
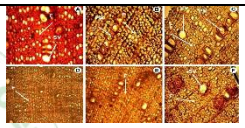
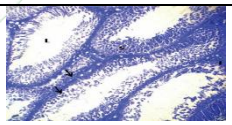
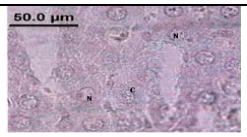
Histology is a study of cells and tissues of animals and plants through staining and cutting are then examined under a microscope (electron or light microscope). The histological studies used in forensic, investigation, autopsy, diagnosis, and education. In pharmaceutical, histology used in treatments to search for diseased tissue to aid treatment[1]. Histological staining is one of the technical processes in the preparation of tissue samples by coloring using histological staining to assist in the study[2]. Histological staining techniques have developed very rapidly both through chemical, molecular biological tests, and immunological techniques collectively and greatly facilitate the study of tissues and organs[3].

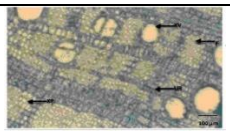
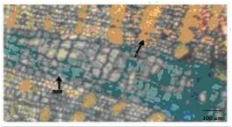
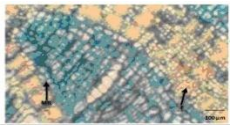
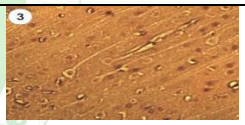
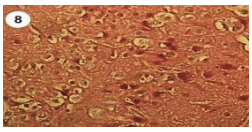
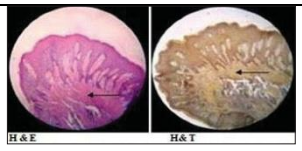
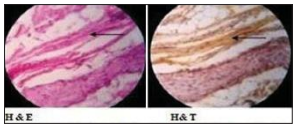
Ordinary dyes can be classified into four groups, namely synthetic dyes, dyes made similar to natural dyes, inorganic dyes and natural dyes[4]. Synthetic dyes commonly used as tissue dyes such as safranin-O and methylen blue (MB) are relatively easy to use in microscope inspections. However, the use of these dyes has a negative effect on the human body, where safranin-O is one of the cationic dyes whose waste can cause dangerous contamination in pharmaceuticals and textile factories, besides safranin-O dyes can cause negative effects on human health such as skin allergies[5]. In addition, MB causes cardiovascular disorder, dizziness, fever, headache, skin problem and anaemia[6]. MB is also carcinogenic and very difficult to decompose[6,7.]An alternative solution to overcome this problem is to use natural dyes that are more eco friendly, non-carcinogenic, non-toxic and biodegradable[8]. Plants have great potential as a source of natural dyes, especially in the roots, bark, leaves, fruit, wood, seeds, and flowers[9,10,11]. The use of natural strains do not cuase harms, cheap, non-allergic, non-toxic, and eco friendly[8].

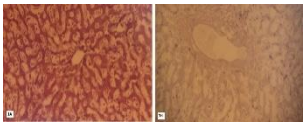
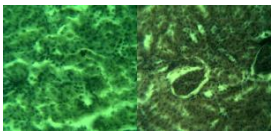
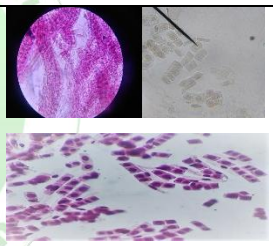
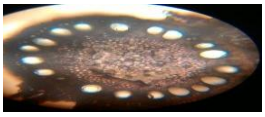
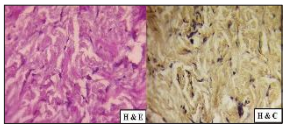
METHODS

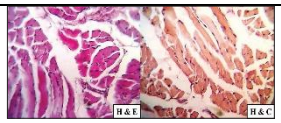
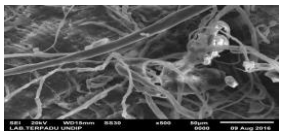
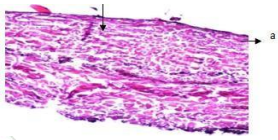
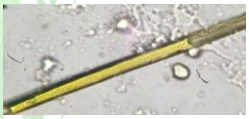
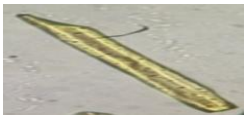
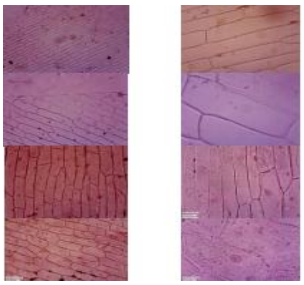
The author created this article review by conducting literature studies. The primary data was collected from international journals in the last ten years (2010-2020). The works of literature were collected from trusted online journal sites such as the digital library, Science Direct, Pub Med, NCBI, Researchgate, Google scholar, and other E-resource with keyword “Natural dyes”, “Histology”, and “Tissue”.

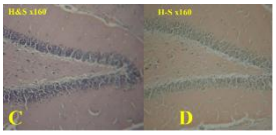
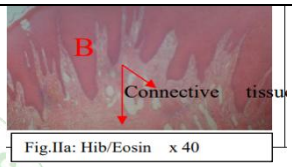
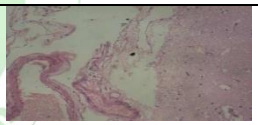
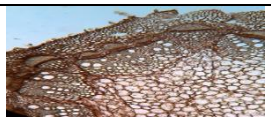
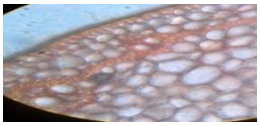
RESULTS OF UTILIZATION OF NATURAL DYES USED FOR ORGAN HISTOLOGY

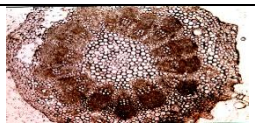
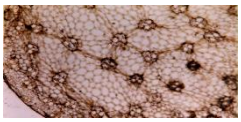
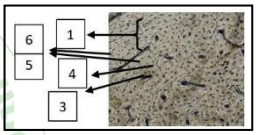
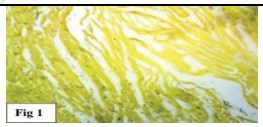
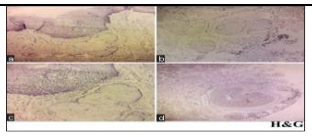
S. No.	Plant Species	Plant Parts	Parts tested	Result	Result Image	Countries	Author
1	*Erythrina crista-galli*	Flower	The cross section of the *piper betle* L.	The *Erythrina crista-gali* flower tested against the cross-section of *piper betle* stems provides good staining quality at a concentration of 70%.	  Concentration of 70% in February and July	Indonesia	12
2	*Indigenous Berberis Pachyacantha Kochne*	Plant	The cross section of *Zea mays* and *Helianthus annuus*	*Berberis pachyacantha kochne* in ethyl has proven to be highly selective.	 (A).Cross-section of *Helianthus annuus L.*, stained with extracts of *Berberis pachyacantha kochne*.	Pakistan	13
3	*1. Enantia chloratha* *2.Harugana madagascariensis* *3.Hibiscus sabdariffa* *4.Sarcocephalus latifolius* *5.Sphenocentrum jollyanum* *6.Sorghum bicolor*	bark, flower petals, roots, leaf sheath	Wood anatomy	The phytodyes used are highly selective in providing color to fibers and other lignified cells and will be even better when used with dyes that have an affinity forthin-wall cells (eg parenchyma cells) except for *Hibiscus sabdariffa*.	 (A).T.S. single stain wood cross section with Phytodyes (X400).	Nigeria	14
4	*Lonchocarpus Cyanescens*	Leaves	Testicular tissue	The staining used to stain the structures on the testes to a blue color in the optimal staining intensity was achieved at a staining time of 5 minutes.	 (A).Time: 5 minutes Figure 1: Photomicrograph of testes stained with *Lonchocarpus cyanescens.*	Nigeria	15
5	*Syzygium cumini*	Fruit	Rat liver tissue	The rat liver tissue can be colored by natural dyes from black plums, namely the nucleus and cytoplasm of the liver cells.		Thailand	16
6	*Allium cepa*	Onion peel	Tissue	The cytoplasm of cells and connective tissue was stained with a color that appears reddish to yellowish brown in color.		Nigeria	17
7	*Hibiscus Sabdariffa*	Flower	Tissue skin biopsy	The *Hibiscus Sabdariffa* solution produced the best staining in 5% solution and the		Sudan	11

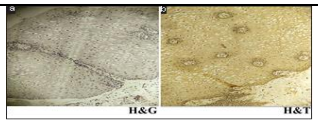
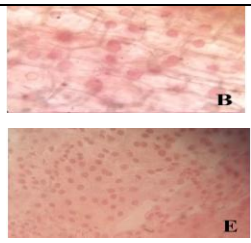
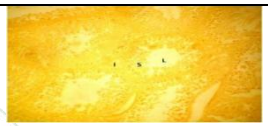
				staining time was 60 minutes.			
8	*1.Bixa orellana* *2. Pterocarpus osun* *3. Curcuma domestica* *4.Lonchocarpus cyanescens*	The seeds Dry wood core Rhizome Young leaves	*Cola gigantean wood*	The dye extract from *Bixa orellana* gives a striking orange color to the fibers and vessels. Meanwhile, the dye extract from *Curcuma domestica* is discriminatory in giving the fibers a brilliant yellow color, The dye extract from *Lonchocarpus cyanescens* easily absorbs moisture from the ipso facto atmosphere and is thought to be rich in sugar content. Also the dye extract from *Pterocarpus osun* gives its red color to all cells except fibers and blood vessels. In addition, the dye extracts from *B.orellana*, *C.domestica* and *P.osun* were specific when used in double staining with Alcian blue.	   The cross sections of *Cola gigantea* bark were stained with dye extracts of *Bixa orellana*, *Curcuma domestica*, *Pterocarpus osun* and *Alcian Blue*.	Berlin Heidelberg	18
9	*Black* Mulberries *(Morus Nigra)*	Fruit	Nerve Rat tissue staining	The hippocampus and cortex neurons are stained with dark brown. The hypothalamus is stained brown and neurons are observed as light brown and the staining reaction is poor.	 (A).The histological architecture of the rat cortex was stained with mulberry extract. 200 X.  (B). The histological architecture of the rat hippocampus was stained with mulberry extract. 200 X.		19
10	*Curcuma longa L.*	Rhizome	Epithelial tissue, keratin, collagen fibers, muscle, adipocytes, blood vessels and red blood cells (RBC), cartilage and bone	Turmeric can be used as a stain after hematoxylin, its staining ability is the same as eosin staining with special affinity for collagen and muscle fibers.	 (A). Collagen photomicrographs showed a comparison of eosin and turmeric staining ability at × 10  (B). Muscle photographs showed a comparison of eosin and turmeric staining ability × 40.	India	20

11	*Ceratonia Siliqua Bark*	Bark	Liver, lung and kidney tissue.	Bark extract from *Ceratonia siliqua bark* dyes collagen fibers, red blood cells and muscle fibers by giving it a light brown color 12 within 15 minutes. The staining can be affected by pH and the environment because the bark extract of *Ceratonia siliqua* combined with haematoxylin provides poor tissue architecture when compared to eosin-combined haematoxylin dyes.	 (A). (a and b): The human liver control section (H&E x100) showed normal characteristics of Haematoxylin and Eosin (3A) staining, and the human liver portion (H & *Ceratonia siliqua* x100 bark extract) showed poor cytoarchitectural differences (3B).	Nigeria	21
12	*Cola acuminata*	Seed	Rat tissue	*Cola acuminata* and haematoxylin extracts could stain then ucleus and cytoplasmic tissue and it color was bluish and yellowish.	 (A). Rat Kidneys with Haematoxylin & Kola Extract x400). (B). (Haematoxylin & Eosin x400)	Nigeria	22
13	*Hylocereus polyrhizus*	Pulpy	*Allium ascalonicum* chromosome	Mitotic cell chromosomes stained red with 1% aceto-orcein were used as positive control.	 1.Chromosome staining onion *Allium ascalonicum* with a ratio of natural dyes using a magnification of 40x:A. The squashing method with the ratio A contains 2:1 (w/v), the asquashing method with 1% aceto-orcein as a positive control.	Indonesia	23
14	*Hylocereus costaricensis*	Pulpy	Plant tissue	*Hylocereus Costaricensis* fruit peel extract can provide color with the most effective concentration was 60% higher percentages than other concentrations.	 The root of *Zea mays* L. with dragon fruit peel extract staining 60%	Indonesia	24
15	*Curcuma longa*	Rhizome	Epithelial tissue, keratin, collagen fibers, muscle, adipocytes, Blood vessels and red blood cells (RBC), cartilage and bone	Curcumin's ability to staining these structures by imparting a distinct, yellowish hue and manner, a special affinity for collagen and muscle fibers.	 (A).Collagen photomicrographs show comparisons eosin and turmeric staining ability (× 40).	India	25

					 (A). Muscle photomicrographs showed the ratio of eosin and turmeric staining ability (× 40).		
16	*Rhizopora sp*.	Bark	Tilapia skin tissue	Testing the mangrove peel solution with a soaking time of 90 minutes. The SEM test results of natural dyes for mangrove bark, collagen structure, have empty spaces which tend to be hollow and un even. So tha it causes the low physical quality of tilapia.	 (A). Cross section of tilapia skin collagen with mangrove bark dye for 90 minutes soaking.	Indonesia	26
17	*Intsia bijuga*	Wood	Skin tissue	The results showed that at a concentration of 25% it was seen that the color more evenly seeped into collagen fibers and had a better structure of skin tissue.	 (A). 25% Merbau solution	Indonesia	27
18	*Curcuma domestica* Val.	Turmeric rhizomes	Melinjo plant tissue	The staining used for plant tissue maceration is turmeric filtrate and turmeric filtrate mixed with lime / Ca (OH) 2 water. The color seen on the plant tissue preparations using turmeric filtrate alone looks yellow but less bright. Meanwhile, the preparations that use turmeric filtrate dye mixed with lime give a brighter yellow color.	 (A). Sklereid Melinjo using turmeric + Ca (OH) 2 filtrate dye, magnification 400x.  (B).Melinjo tracheids using turmeric + Ca (OH) 2 filtratedye, microscope magnification 1000x.  (C). Melinjo trachea using turmeric + Ca (OH) 2 filtrate dye, microscope magnification 400x.	Indonesia	28
19	*Clitoria ternatea*.L	Flower	Plant tissue (*Allium cepa* L*)*	With the addition of 4 grams, 6 grams, 8 grams and 10 grams of flower telang added with a 1: 1 citric acid solution, the staining began to look good. The color produced in the telang flower extract was almost the same as the		Indonesia	29

				synthetic dye / safranin after being dripped on the preparations, the color appeared purplish red on the observed parts of the cell wall, cytoplasm and cell nucleus.			
20	*1. Hibiscus sabdariffa* *2. Sorghum bicolor*	1. Dried Leaves 2. Stems	Wistar rat brain tissue	All the photomicrograph groups displayed the same hippocampal cells and were almost indistinguishable. The layers of neurons and glial cells can be colored well. But the stains differ greatly from the hippocampal component which is comparable to controls.	 The histomorphological features of the hippocampal layer, neurons and glial cells were clearly preserved in all groups.	Nigeria	30
21	*Hibiscus sabdariffa*	Dried leaves	Skin tissue	Staining with *Hisbiscus-eosin* gave a blackish blue color, while staining with H&E staining gave a purplish blue color and cytoplasmic components were red with the use of Hisbiscus-eosin for H&E.		Nigeria	31
22	*Hibiscus sabdariffa*	Dried leaves	Cerebellum, cerebrum, and pons tissue	The brain tissue is well stained when compared to standard synthetic hematoxylin and eosin dyes. The nucleus is dark purple and red stains the blood cells while the cytoplasmic components are stained with a light pink color.	 Ia: *Hibiscus* / Eosin Cerebrum Mag x 400. Nucleus: dark purple, cytoplasm: pink	Nigeria	32
23	*Tectona grandis*	Young leaves	Plant tissues	Young teak leaf filtrate dye can absorb and color tissue in *Pluchea indica* stem preparations, *Glycine max* root preparations, *Panicum sp* stem preparations, and *Ordchidae* root preparations such as epidermis, parenchyma, phloem, xylem, sclerenchyma, and also pericicles.	 Cross section of *Plucea indica* stem  Cross section of *Panicum* sp stem	Indonesia	33
24	1. *Hisbiscus* 2. *Sorghum bicolor*	1. Flowers 2. Trunk	Liver and kidney tissue	The use of *Hibiscus-Sorghum* color combination, the nucleus components appear	Image 1a: *Hibiscus / Sorghum*. Kidney showing dark purple nucleus and prominent Bowman's capsule x 400.	Nigeria	34

				dark purple, the cytoplasmic components appear light brown.	 Image Ib: *Hibiscus / Sorghum*. Liver shows nucleus: dark purple and cytoplasm: light brown x 400.		
25	*Lawsonia inermis linn*	Dried leaves	Aniospermic stem tissue	The dye extract from *Lawsonia inermis* which is dissolved in ethyl alcohol and water can be used effectively to stain tissue in lignified plants if used in a single stain.	 A. *Helianthus annuus* L. stem cross-section, stained with *Lawsonia inermis* L. extract, in water.  B. *Zea mays* L. stem cross sections, stained with *Lawsonia inermis* L. extract, in ethyl alcohol.	Pakistan	35
26	*Clitoria ternatea L.*	Flower	Bone scrub preparations	Natural staining of the telang flower with a ratio of 1:10 and the resulting image was good, the parts that were clearly visible were the havers system, canalis havers, and osteocytes. The less obvious parts are lacunae, lamellae, and canaliculus.	 Preparation of chicken femur with natural staining of telang flowers 1:10	Indonesia	36
27	*Zingiber officinale*	Rizhome	Muscle fibers and cytoplasm of Wistar rats	The solution of *Zingiber officinale* extract used for staining canstain muscle fibers and cytoplasm with a yellow colorand a dark green core in it, when used as a counter stain for hematoxylin for 4 minutes. The staining reaction can be observed to be similar to that of eosin, except for its yellow-green color.	 Figure 1: Parts of heart tissue stained with 90% ethanol extract of *Z.officinale*, muscle fibers stained yellow and core dark green.	Nigeria	37
28	*1.Zingiber officinale Roscoe* *2. Curcuma longa L*	Rizhome	Tissue	Compared with *Curcuma longa*, *Zingiberis officinale* showed better staining intensity and specificity on the cytoplasm and connective tissue components. And shows a significant difference. The long shelf life of *Zingiberis*	 Figure 1: Photomicrograph showing epithelial cytoplasmic stains (a × 10), connective tissue components such as nerve bundles, muscle fibers (b × 20), sebaceous glands (c × 40), and blood vessels (d × 20), using stain *Z Officinale*.	India	38

				officinale is also better than *Curcuma longa*.	 Figure 2: Photomicrograph showing the comparison of cytoplasmic stains on the epithelial layer using *Z. officinale* (a) and C. longa (b) stains at × 20.		
29	1.*Melastoma malabathricum* 2. *Ipomea batatas var*	1. Fruit 2. Tubers	*Allium cepa* roots	The staining time of 90 minutes is the best result obtained from the preparation. And the comparison of *Allium cepa* mitotic preparations has a very good quality when colored with senduduk fruit extract while sweet potato extract provides a fairly good staining quality.	 Figure 1. Staining with senduduk extract: B. Staining for 90 minutes. Purple sweet potato extract staining: E. Staining 90 minutes	Indonesia	39
30	*Curcuma Longa*	Rhizome	Testes	The use of *Curcuma longa* dye for 15 minutes at a concentration of 0.2 g / ml can clearly stain the testes on the seminiferous epithelial tissue and the intersitum yellow. *Curcuma longa* also provides a great stain counter for Haematoxylin.	 Figure 1: Photo micrograph of testes stained with *Curcuma longa* at a concentration of 0.2 g / ml for 15 minutes.	Nigeria	40

DISCUSSION

The application of natural dyes from plants for staining of various biological tissues from an alternative source will decrease the expense for purchasing the synthetic dye and reduce their effects on human and environment[16,18]. Based on the results of the utilization of natural dyes used for organ histology, each plant used as natural dyes has the ability to color tissue with different qualities. The natural dye of the *Erythrina crista-gali* flower tested against the cross-section of *piper betle* stems provides good staining quality at a concentration of 70%. The coloring ability of *Erythrina crista-gali* flowers is caused by the presence of anthocyanin compounds[12]. In addition, the *berberis pachyacantha kochne* plant extracted with ethyl was found to contain curcuminoids which were shown to selectively color the cross-section of the stems of *Zea mays* and *Helianthus annuus*[13]. Meanwhile, the leaves of the *Lonchocarpus cyanescens* plant which are used to color testicular tissue contain several compounds such as saponins, tannins, flavonoids and alkaloids. But the compounds that play an important role in giving color are tannins and flavonoids so that the testicles appear blue[15]. The fruit of *Syzygium cumini*, which was tested to coloring the liver tissue of rats, it showed a color change in nucleus and cytoplasm, because the fruit contained tannin and anthocyanin compounds that could give color[16]. The natural dye for the skin of the *Allium Cepa* tube which is used to color the tissue contains saponin and tannin compounds that give color changes to the cytoplasm of cells and connective tissue so that it looks reddish to yellowish brown color[17].

Natural dyes from the rhizome of *Curcuma longa* that are used contain curcuminoid compounds that have good coloring ability after hematoxylin, especially against collagen tissue and muscle fibers[20,25]. *Cola acuminata* seeds containing anthocyanin and tannin compounds used to color rat tissue in the nucleus and cytoplasm give a bluish and yellowish color[22]. Natural dye from the pulp of *Hylocereus polyrhizus* containing the compound betasianin was used to staining the chromosomes of *Allium ascalonicum* in red[23]. In addition, the skin of *Hylocereus costaricensis* which contains anthocyanin compounds can also be used to color plant tissue, with the most effective concentration at 60%[24]. This staining is to facilitate observation of cells or tissues under a microscope, because the substance has a selective affinity for cell organelles[10]. *Curcuma domestica* rhizome which contains curcuminoid compounds was used to color plant tissue on melinjo stems, and gives a brighter color when mixed with Ca(OH)2[28]. The higher the concentration of Ca (OH) 2 added, will affect the strength of the given color[42]. There is also a tissue

staining using dried leaves *Hisbiscus sabdariffa*-eosin-containing compounds anthocyanins for coloring skin tissue. *Hibiscus sabdariffa* without combination is used to color the cerebellum, cerebrum and nucleus with different colors[31,32]. Young leaves of *Tectona grandis* are also used as natural dyes because it contain anthocyanins for staining plant tissue in the epidermis, parenchyma, phloem, xylem, sclerenchyma and perisicles which can absorb and color tissue[33]. *Melastoma malabathricum* fruit that contains flavonoid compounds, saponins, tannins and *Ipomea batatas* tubes contains anthocyanin and tannin compounds. It used as natural dyes to give color to the *Allium Cepa* root tissue with the best coloring results obtained for 90 minutes of staining. The rhizome of *Curcuma longa* which contains saponins, tannins, flavonoids, and alkaloids with a concentration of 0.2 g / ml which is used for staining the testes, gives a yellow color[40].

CONCLUSION

Some plants that used for natural dyes in the histology of animal and plant tissues, it contains chemical compounds that can provide colors such as flavonoids, alkaloids, saponins, tannins, anthocyanins, betasianins, and curcuminoids.The resulting dye influenced by pH, concentration, staining time, method and solvent used in the staining.

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