An overview of research on plant based natural dyes in Nepal: scope and challenges

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ABSTRACT
The worldwide demand of natural dyes is of great interest due to the increased public awareness about the atmospheric and environmental pollution caused by the commercially available synthetic dyes. Nepal being wealthy in flora, would be fine research laboratory land for the plant based natural dyes. Among most of the natural dyes, plant-based dyes/pigments have wide range of applications in fabric, food, drug coloring, therapeutic values and also in solar cells in presence of different mordants. The use of mordant is inevitable during natural dyeing process in order to improve the fastness properties on fabrics, foods and drugs by forming a co-ordination complex with dye. In this article, a short overview of plant based natural dyes extraction applications and their scope and limitations will be discussed with special reference to Nepal. In the present review, the green methods of dye extraction, and dyeing technologies will be discussed, and the research fields based on natural dyes will be explored. Some of the natural dyes has also shown the antimicrobial, antioxidant, antifungal properties and hence are also discussed with biomedical applications.

Keywords: Natural dyes, mordant, textile, antimicrobial activity, synthetic dyes

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INTRODUCTION
Globally, the demand for natural dyes is significantly increasing due to its cheaply available, widely applicable, and extraction that is carried out by green methods from natural resources (Siva, 2007; Kadolph, 2008; Jaffer et al., 2019). Dyes are interrelated to the pigments in some way but these two are different terms. Dyes, also known as colorants in which the coloring matter is dissolved in liquid, are absorbed into the material to which they have to be applied. On the other hand, pigments consist of extremely fine particles of ground coloring matter...
suspended in a liquid which forms a paint film that bonds to the surface it is applied to (Chaitanya, 2014).

Nepal has a rich biodiversity with varieties of natural products. Dyes are the most vital product which is obtained from plants, minerals, animals, insects, etc. (Paudel et al., 2011, Kamel et al., 2005). Natural dyes are more popular due to its nonhazardous and wide applicability such as pharmaceuticals, food, cosmetics, leather, and in different art of dyeing (Kanchana et al., 2013; Tayade et al., 2016). The majority of dyes do not interact directly with the materials and are required a mordant to fix to the fabric and prevent the color from either washing out or fading with exposure to light. The mordant enhances light fastness, wash fastness, dry fastness, and color intensity by the formation of the complex with dye and fixing the natural colorant on fibers. Alum, potassium dichromate, ferrous sulphate, copper sulphate, tannin, and tannic acid are some of the important mordant used in textile dyeing (Siva, 2007; Prabhu, 2012).

The synthetic dyes are environmentally toxic and hazardous to health over natural dyes. The synthetic dye is obtained from the different inorganic metal complexes whereas, natural dye is obtained from the natural plants, insects and minerals (Singh et al., 2017; Singh & Singh, 2018; Wangatia, 2015). Since, long time ago, in the ancient time of human civilization, mineral, plant and animal products were the main sources of dyes and drugs with their excellent therapeutic properties (Bhuyan et al., 2016). The synthetic colorants possess various side effects and radiation hazards (Chengaiah et al., 2010; Samata & Agarwal, 2009). Turmeric, a naturally occurring yellow dye which revitalizes the skin, is a powerful antiseptic, while indigo gives a cooling sensation.

Green methods for plant dye extraction have been studied and classified as medicinal importance as well as some of these have recently been shown to possess antimicrobial, antifungal, antioxidant and anticancer activities (Hussein et al., 1997). Curcuma longa, Punica granatum L, lawsona from Lawsonia inermis L, have been reported for the antimicrobial, antibacterial and antifungal activities (Paudel et al., 2011).

The focus of this review is to study and discuss the various dye yielding plant parts, extraction methods and dyeing technologies that are traditionally in practices in Nepalese culture, and then explore the new ideas that could make the dye extraction and dying methods safer, easier, durable and sustainable with respect to human health and the environment. Further, the review will also emphasis the advancement and opportunities of using natural mordants to replace the synthetics in textile/fabric, drugs, food coloring, solar cells, and as therapeutic agents.

**Extraction and separation of natural dyes**

Plant dyes are extracted in various solvents but alcoholic or aqueous are common. The temperature and way of extraction depend on the nature of dyes to be extracted and the solvent used. The alcoholic extraction usually occurs in ethanol as solvent at 50°C whereas, water is used as solvent at 70-80°C for 2 hours in aqueous extraction (Kannanmariyani et al., 2015; Khan et al., 2018; Das et al., 2016). Natural dyes such as curcumin, dendranthema, lawsonia, betanines, etc. are obtained from Curcuma longa L, Dendranthema grandiflora, Lawsonia inermis and Beeta vulgaris, respectively (Hasan et al., 2014; Chengaiah et al., 2010).
2010; Das et al., 2016; Kannanmarkani et al., 2015; Aura et al., 2011). The component of extracted curcumin dye were identified and tested for the purity by thin layer chromatography (TLC) and is separated in large scale by column chromatography (CC). The retention factor (Rf), calculated by equation 1 helps to check the purity and identify the component of curcumin dye (Hasan et al., 2014).

$$R_f = \frac{\text{Distance travelled by component}}{\text{Distance travelled by solvent}}$$  

Figure 1 shows the different retention factor of curcumin dye extracted from *Curcuma longa* L. The Rf1 is very high as compared to Rf2 and Rf3. This shows that component 1 moves faster as compared to component 2 and component 3 in mixture of curcumin dye (Hasan et al., 2014).

Figure 1: Calculation of Rf from developed in TLC plate (Hasan et al., 2014)

FTIR (Fourier-transform infrared spectroscopy) has proven a valuable tool for the characterization and identification of compounds or functional groups present in an unknown mixture of plants extract. For most common plant compounds, sample preparation for FTIR analysis involves the various ways. For liquid samples, one drop of sample is placed between two plates of sodium chloride and it forms thin film between the plates (Sasidharan et al., 2011). The spectral data of *Lawsonia inermis* (henna) leaves has wide band intensity at 3400 cm\(^{-1}\) indicate the presence of hydroxyl groups (O-H), absorption peak at 2924 cm\(^{-1}\) represents the C-H stretching, absorption at 2363 cm\(^{-1}\) indicate the stretching of (C=C), also the carbonyl group (C=O) absorption at (1728-1625 cm\(^{-1}\)) and absorption at 1420, 1041, 633 cm\(^{-1}\) for the C-H bending, etheral O-CH\(_3\) stretching and bending vibrations respectively (Musa & Gasmelseed, 2012). The FTIR spectra of *Lawsonia inermis* (henna) leaves extract is presented in Figure 2.
Figure 2: FTIR spectrum of *Lawsonia inermis* (henna) (Musa & Gasmelseed, 2012)

A list of commonly used dye yielding plants is given in the Table 1 along with its parts used, color obtained and medicinal uses.

**Table 1: Important dye-yielding plants with their medicinal value**

| S.N | Plant name | Family       | Parts used | Color obtained | Medicinal use                                           | References               |
|-----|------------|--------------|------------|----------------|--------------------------------------------------------|--------------------------|
| 1.  | *Abies spectabilis* (Himalayan Fir) | Pinaceae     | Cone       | Purple/violet  | Used for curing cough                                  | (Siva, 2007)             |
| 2.  | *Acacia catechu* (Catechu) | Mimosaceae   | Bark       | Brown/Black    | Used medicinally for sore throat and cough             | (Shinda et al., 2019)    |
| 3.  | *Adhatoda vasica* Nees (Malbar nut) | Acanthaceae  | Leaf       | Yellow         | Used in bronchial infection                            | (Singh & Srivastava, 2017)|
| 4.  | *Aegle marmelos* L. (Bael) | Rutaceae     | Fruit rind | Yellow Pale yellow | Used as digestive and for curing stomachache diarrhea | (Kadam et al., 2018)     |
| 5.  | *Ageratina adenophora* (Sticky snakeroot) | Asteraceae   | Leaves     | Yellow         | Regulating menstruation and activating blood, detoxifying and eliminating swelling | (Fan et al., 2018)       |
| 6.  | *Allium cepa* (Onion) | Liliaceae    | Fruits and leaves | Yellow         | Stimulate appetite, help digestion, sterilization, anti-cold. | (Vankar & Shanker, 2009) |
| 7.  | *Aloe barbadensis* L. (Aloe vera) | Asphodelaceae | Whole plant | Red            | Used in liver and spleen ailments and for eye infections, useful in X-ray burns | (Annapoorani & Divya, 2015) |
| No. | Species Name | Family | Part Used | Color | Uses |
|-----|--------------|--------|-----------|-------|------|
| 8.  | Azadirachata indica (Neem) | Meliaceae | Bark | Brown | Skin disorders, leaves considered as anti-septic (Bukhari et al., 2014) |
| 9.  | Barleria prionitis L. (Porcupine Flower) | Acanthaceae | Flower | Yellow | Treating fever, respiratory diseases, toothache, joint pains (Pal et al., 2018) |
| 10. | Berberis aristata (Indian barberry) | Berberidaceae | Bark | Yellow | Antibacterial, antidiarrheal and anticancer and it is also used in the treatment of ophthalmic infections (Semwal et al., 2012) |
| 11. | Betula utilis (Bhoj patra) | Betulaceae | Tree gum | Brown | Infusion of bark is aromatic and antiseptic; also used as a carminative (Siva, 2007) |
| 12. | Bute monosperma (Palas) | Fabaceae | Flower | Yellow/orange | Astringent, antidiarrheal, antidyserteric, purgative and anthelmintic (Singh et al., 2017) |
| 13. | Calendula officinalis (Marigold) | Asteraceae | Flower | - | Act as powerful antioxidant and can be used in skin infections (Chalakkal et al., 2019) |
| 14. | Camellia sinensis (Tea) | Theaceae | Leaves | Brown | Used to reduce inflammation and green tea is used as anticancer (Kaur, 2015) |
| 15. | Capsicum annuum L. (Chili) | Solanaceae | Fruits | Red | Digestive, carminative, stimulant, cardiotonic, antipyretic (Kulkarni et al., 2011) |
| 16. | Cassia fistula L. (Golden shower) | Fabaceae | Bark | Brown | Used as antidiabetic, hepato protective, anticancer, antibacterial, wound healing, anti-itching, anti-ulcer, etc. (Mongkholrattanasit et al., 2016) |
| 17. | Castanopsis indica (Indian Oak) | Fagaceae | Bark | Brown | - | - |
| No. | Species Name     | Family       | Part(s)      | Color(s)       | Uses                                                                 | Reference(s)                  |
|-----|-----------------|--------------|--------------|----------------|----------------------------------------------------------------------|--------------------------------|
| 18. | *Cedrela toona* | Meliaceae    | Flower, seed, leaf | Yellow, red    | Bark used for chronic dysentery of infants and also in external application of ulcer | (Siva, 2007)                  |
| 19. | *Carthamus*     | Compositae   | Flower       | Red, yellow    | Oil applied to sores and rheumatic swelling; also used in case of jaundice | (Chengaiah et al., 2010)      |
| 20. | *Crocus*        | Iridaceae    | Flower       | Yellow, orange | Used as sedative and emmenagogue                                      | (Chaitanya, 2014)             |
| 21. | *Curcuma*       | Zingiberaceae| Rhizome      | Yellow         | Anti-oxidant, anti-inflammatory, anti-cancer, anti-viral, anti-fungal & anti-bacterial effects | (Chaitanya, 2014)             |
| 22. | *Cynadon*       | Poaceae      | Aerial part  | -              | Anti-microbial, anti-viral, anti-diabetic, antiulcer, analgesic and anti-pyretic properties | -                             |
| 23. | *Embellia*      | Myrsinaceae  | Fruit        | Red            | Antibacterial, abdominal disorders, lung diseases, analgesic, anti-inflammatory, antioxidant | (Gupta, 2019)                 |
| 24. | *Eugenia jambolana* | Myrtaceae    | Bark/Leaf    | Red            | Antibacterial, antifungal, anti-viral, anti-inflammatory, cardio-protective, anti-allergic, anticancer, chemo-preventive | (Garcia et al., 2003)         |
| 25. | *Gardenia*      | Rubiaceae    | Fruit        | Yellow         | Clearing away heat, purging fire, cooling blood                       | (Hong et al., 2015)           |
| 26. | *Gossypium*     | Malvaceae    | Flower       | Yellow         | Gastro-intestinal issues, such as hemorrhages                         | (Sabane, 2018)                |
| No. | Plant Name                  | Family            | Part Used | Color     | Uses                                                                                                               |
|-----|-----------------------------|-------------------|-----------|-----------|---------------------------------------------------------------------------------------------------------------------|
| 27  | *Hibiscus rosa-sinensis*    | Malvaceae         | Flower    | -         | Used to reduce swelling pain (Ramprasath et al., 2017)                                                             |
| 28  | *Hibiscus sabdariffa* L.    | Malvaceae         | Fruit     | Red       | Clearing away summer heat, eliminate fatigue, lowering blood pressure, relieving asthma, detoxification (Yilmaz & Bahtiyari, 2017) |
| 29  | *Hypericum cordifolium*     | Hypericaceae      | Leaves    | Black     | Used as anxiety, depression, insomnia, water retention, and gastritis.                                               |
| 30  | *Impatiens balsamina* L.    | Balsaminaceae     | Flower    | Red       | Promoting blood circulation to reduce swelling and treating traumatic injury (Wahyuonoa & Risantob, 2012)             |
| 31  | *Indigofera tinctoria* L.   | Fabaceae          | Leaf      | Blue      | Extract used in epilepsy and other nervous disorders. Roots used in urinary complaints as hepatitis (Wahyuningsih et al., 2016) |
| 32  | *Jatropha curcas* L.         | Euphorbiaceae     | Bark/Leaf | Blue      | Used as scabies, ringworm, gonorrhea, dysentery, diarrhea (Babel et al., 2013)                                      |
| 33  | *Juglans regia* (Walnut)    | Juglandaceae      | Bark      | Brown, black | Antibacterial, Antiviral, Anti diabetics, Anti - cancer as well as cardiovascular benefits (Mirjilalili & Karimi, 2013) |
| 34  | *Lawsonia inermis* (Henna)  | Lythraceae        | Leaf      | Orange    | Anti-diarrheal, anti-dysenteric, emmenagogue liver tonic and anti-fungi (Chaitanya, 2014; Kannanmarikani, 2015)     |
| 35  | *Mallotus philippiensis* (Kamala tree) | Euphorbiaceae     | Fruit    | Red       | Employed as an antioxidant and for cutaneous infection (Kumar et al., 2016)                                         |
| 36  | *Michelia champaka* L. (Champa) | Magnoliaceae     | Flower    | Yellow    | Used as tonic for stomachache and carminative, used (Armiyanti et al., 2010)                                        |
in dyspepsia, nausea and fever, also useful as a diuretic in renal diseases

| No. | Plant Name          | Family   | Part Used      | Color          | Use                                                                 | Reference                          |
|-----|--------------------|----------|----------------|----------------|----------------------------------------------------------------------|------------------------------------|
| 37  | *Morus alba*  | Moraceae | Bark and leaves | -              | Exhibit antibacterial activity against food poisoning microorganism | (Kumari et al., 2016)              |
|     | (White mulberry) |          |                |                |                                                                      |                                    |
| 38  | *Myrica esculenta* | Myricaceae | Bark          | Brown          | Used to treat asthma, cough, chronic bronchitis, ulcers, inflammation, anemia, fever, diarrhea, and ear, nose, and throat disorders. | (Jangwan et al., 2007)              |
|     | (Bayberry)         |          |                |                |                                                                      |                                    |
| 39  | *Ocimum tenuiflorum* | Lamiaceae | Leaves        | Reddish brown | Used as anticancer, hypoglycemic, anti-spasmodic and as a hypotensive | (Prabu & Anabarasan, 2015)         |
|     | (Tulsi)            |          |                |                |                                                                      |                                    |
| 40  | *Phyllanthus emblica* | Phyllanthaceae | Bark          | Yellow, green, black | Bark of this plant rich in tannin so used for dysentery, diarrhea, liver disease and cough | (Sakthivel, 2015)                  |
|     | (Amala)            |          |                |                |                                                                      |                                    |
| 41  | *Pistacia intergerrima* L. | Anacardiaceae | Flower, leaf | Yellow         | Useful for asthma and other respiratory tract disorders and also for dysentery | (Siva, 2007)                       |
|     | (Kaakadsinghi)     |          |                |                |                                                                      |                                    |
| 42  | *Pterocarpus santalinus* L. | Fabaceae | Wood          | Red            | Hepato-protective                                                    | (Azamthulla et al., 2015)          |
|     | (Raktachandan)     |          |                |                |                                                                      |                                    |
| 43  | *Punica granatum* | Punicaceae | Bark          | Pale yellow    | Antibacterial, Antiviral, Cardiac, Demulcent, Stomachic &Vermifuge  | (Khan et al., 2018; Adeel et al., 2009) |
|     | (Pomegranate)      |          |                |                |                                                                      |                                    |
| 44  | *Rubia cordifolia* L. (Indian madder) | Rubiaceae | Root          | Red            | Antitussive, astringent, diuretic, expectorant and styptic          | (Yusuf et al., 2011)               |
| 45  | *Solanum*          | Solanaceae | Fruits        | Red            | Anti-bacterial                                                      | (Tayade et al., 2018)              |
Most of the natural dyes are obtained from different parts of plants such as leaves, flowers, bark, fruit, and roots. Table 1 represents the different dyes yielding plants and their medicinal values. There is a traditional use of dye yielding plants for medicinal purposes. Plants like turmeric, saaj, tulsi, amala, khayer, neem, etc. are traditionally used for medicine. They can be used as anti-oxidant, anti-inflammatory, anti-cancer, anti-viral, anti-fungal, anti-bacterial, hyperglycemic, anti-diarrheal, anti-spasmodic, skin disorders, etc. (Chaitanya, 2014; Saivaraj et al., 2018; Prabu & Anabarasan, 2015; Sakthivel, 2015; Bukhari et al., 2014).

**Table 1:** Natural dye yielding plants and their medicinal values

| No. | Plant Name                         | Family         | Part | Color    | Use                                                                 |
|-----|-----------------------------------|----------------|------|----------|----------------------------------------------------------------------|
| 46  | *Tagetes erecta* L. (Marigold)    | Asteraceae     | Flower | Yellow   | Used as anti-oxidant, analgesic and anti-bacterial.                   |
|     |                                   |                |       |          | (Farroq et al., 2013)                                               |
| 47  | *Terminalia alata* (Saaj)         | Combretaceae   | Bark  | Red      | Used as antifungal, antioxidant, anti-hyperglycemic, anti-diarrheal  |
|     |                                   |                |       |          | (Saivaraj et al., 2018)                                             |
| 48  | *Terminalia belirica* (Bahera)    | Comberataceae  | Fruit | Blue     | Used to protect the liver and to treat respiratory conditions, including respiratory tract infections, cough, and sore throat. |
|     |                                   |                |       |          | (Srivastava et al., 2015)                                           |
| 49  | *Terminalia chebula* (Black myrobalan) | Combretaceae    | Bark  | Pinkish red | Used as a lotion for sore eyes                                       |
|     |                                   |                |       |          | (Shabbir et al., 2016)                                              |
| 50  | *Zingiber officinale* (Garden ginger) | Zingiberaceae | Rhizome | Brown   | Used as cardio-protective, anti-inflammatory, anti-microbial, anti-oxidant, anti-cancer properties, immune-stimulant etc. |
|     |                                   |                |       |          | (Ajileye et al., 2015; Shakya, 2015)                                 |

Application of natural dyes

Natural dyes have a wide range of applications such as textile/fabric dyeing, drug dyeing, food dyeing and solar cells (Chengaiah et al., 2010; Siva, 2007; Rungruangkitkrai & Mongkholrattanasit, 2012).

Textile/fabric dyeing

Dyes are commonly used in the textile, paper, and leather industries. The synthetic dyes caused water pollution due to the discharge of non-biodegradable colored effluents from textile dye manufacturing (Azanthulla et al., 2015). The natural dyes are important than...
synthetic dyes due to non-allergic, non-toxic and environment friendly (Samata & Agarwal, 2009; Tejavathi & Niranjan, 2018). The mordant used for fabric dyeing are either inorganic type mordant or plant based mordant. In inorganic type mordant various chemicals like alum, potassium dichromate, ferrous sulphate, copper sulphate tannin, tannic acid, etc. are used whereas, in plant based mordant such as mango bark, pomegranate rinds, acorns, oak galls, aloe vera, etc. are used for the fabric dyeing (Singh & Singh, 2018).

Recently, consumers have become aware of sun burn and radiation allergy on skin ageing by acute and chronic reactions and damages (Bhuyan et al., 2016; Rungruangkitkrai & Mongkholrattanasit, 2012). Many of the natural dyes absorb the ultraviolet region and may block the harmful radiations therefore fabrics dyed with such dyes should offer good protection from ultraviolet light (Chattopadhyay et al., 2013; Sarkar, 2004). Fabric dyed with natural dyes had good ultraviolet protective properties and they could absorb about 80% of the ultraviolet rays (Rungruangkitkrai & Mongkholrattanasit, 2012). The natural dyes is also used in fabric dyeing in absence of mordants. The use of natural dye for fabric dyeing in absence of mordants from E. pulcherrima is presented in Figure 3 (Bhandari et al., 2020).

![Figure 3: Crude dye of E. pulcherrima and dyeing on cotton fabric without mordant](Bhandari et al., 2020)

Natural dyes also protect the UV radations in textile industry (Silva et al., 2020). The Ultraviolet protection factor (UPF) for all four dyed samples has a significantly higher value than the undyed sample as shown in Figure 4. Among these four dyed sample, pre-mordant dyed sample has the highest UPF value as well as the lowest ultraviolet transmittance in the UVA region T(UVA)$_{AV}$ and hence design of experiment is to optimize the pre-mordant dyeing conditions for ultraviolet protection (Wang et al., 2009).
The dyeing and ultraviolet protection of silk fabric using vegetable dyes extracted from *Flos sophorae* was found that the aqueous solution of this vegetable dye has excellent thermal stability in acid conditions (Rungruangkitkrai & Mongkolrattanasit, 2012).

**Antimicrobial activity of natural dyes**

Some of natural dyes also possess the antimicrobial and antifungal properties but still a detailed research study has to be done. The three-dye sample were screened for their antimicrobial activity against four bacteria *E. coli* and *Klebsiella sp* (gram negative) and *S. aureus* and *Bacillus sp* (gram positive) (Bhandari et al., 2019; Kanchana et al., 2013). The result below showed that *Punica granatum* (Pomegranate) was effective for antimicrobial activities followed by butterfly pea and marigold among the three dye samples shown in Table 2 (Kanchana et al., 2013).

**Table 2: Antimicrobial activity of natural dyes against test bacteria** (Kanchana et al., 2013)

| Plant species                   | Color obtained | Diameter of zone of inhibition (mm) |   |   |   |
|--------------------------------|----------------|------------------------------------|---|---|---|
| *Punica granatum* (Pomegranate peel) | Pale yellow  | *E. coli* | 8 | 10 | 8 | 12 |
| *Clitoria ternatea* (Butterfly pea) | Blue          | *S. aureus* | 8 | 9  | 7 | 10 |
| *Tagetes erecta* (Marigold)       | Yellow        | *Klebsiella sp* | 6 | 9  | 6 | 9  |
|                                 |               | *Bacillus sp*   |   |    |   |    |

**Antioxidant activity**

The antioxidant activity of plant extracts was determined by using ferric reducing ability of plasma (FRAP) assay as in Table 3. The highest antioxidant activity was shown by dye obtained from *Beta vulgaris* (beet root), followed by *Lawsonia inermis* (henna), *Rosa* (rose) and *Daucus carota* (carrot dye). Higher the FRAP value shows greater antioxidant activity.
Table 3: FRAP values of the dyes with antioxidant activity (Khan et al., 2018)

| Dyes                     | Color obtained | FRAP values |
|--------------------------|----------------|-------------|
| Beta vulgaris (Beet root)| Red            | 2.00        |
| Lawsonia inermis (Henna) | Orange         | 1.99        |
| Rosa (Rose)              | Orange pink    | 1.98        |
| Daucus carota (Carrot)   | Black/blue     | 1.97        |
| Punica granatum (Pomegranate peel) | Pale yellow | 1.94        |

Drug coating

About 60% of the substances that enter in the pharmaceutical industries are plant origin that increases the importance of medicinal plants (Bannerman et al., 1983). Also, these medicinal plants exhibit a wide range of colors where some of these pigments with medicinal values are used as dyes. The environmental friendliness and safer nature of natural dyes makes them more superior as compared to synthetic dyes which consist of harmful chemicals that are allergic and carcinogenic in nature (Shakhatreh, 2013). Normally, in the pharmaceutical industries tablets coating, formulations of tablets, capsules, liquid orals, toothpaste and ointment are colored to increase the aesthetic appearance, prolong the stability, produces standard preparations for identification purposes. The natural pigments, present as active constituent in plants are utilized to meet the demand in medicines. For e.g. Carotenoids are used as vitamin supplements, bixin is applied as natural coloring agents for ointment and plasters. The film coating for the tablets employed chlorophyll. Carotenoids and anthocyanins are considered as basic ingredients (Mohd-Nasir et al., 2018). From the experimental point of view, out of thirteen types of medicinal capsules, it is found that ten types are colored with synthetic dyes and three are colored with mineral dyes as shown in Figure 5. Medicinal capsules derived from synthetic dyes shows the intense color than that of natural dyes (Adriana et al., 2015).

Figure 5: The medicinal capsules submitted to the study of colorants (Adriana et al., 2015)

Therapeutic (medicinal) use of natural dyes

Natural dyes has wide range of medicinal applications due to its antibacterial, antiviral and
antifungal properties (Khan et al., 2018). Different types of medicine are obtained from the natural dyes. Escribano et al., 1996 studied saffron extract and its constituents; crocin, safranal, and picrocrocin from Crocus sativus L. that is used to inhibit the growth of human cancer cells. The dyes obtained from Michelia champaka L. is used in tonic for stomachache and carminative, used in dyspepsia, nausea and fever, also useful as a diuretic in renal diseases (Armiyanti et al., 2010). Many researchers used the various plants species for the extraction of natural dyes which have medicinal applications such as blood circulation (Wahyuonoa & Risantob, 2012), ulcer (Siva, 2007), liver protection (Srivastava et al., 2015), wound healing (Mongkolrattanasit et al., 2016), etc. This results show that natural dyes are wide range of medicinal applications.

**Food coloring**

Turmeric (*Curcuma longa*) contains about 5% of volatile oil, resin and yellow coloring substances known as curcuminoids (Chengaiah et al., 2010). Curcumin is the chief component of curcuminoids used in various food industries for coloring. It is used in dairy products, beverages, cereal, pickles, sausages and also used in hair care and skin care cosmetic products as it is antibacterial in nature (Chaitanya et al., 2014). The chemical structure of curcumin, picrocroin, and lawsone are shown respectively in Figure 6. (Mohd-Nasir et al., 2018; Chengaiah et al., 2010).

![Chemical structure of curcumin, picrocroin, and lawsone](image)

**Figure 6: Chemical structure of (a) curcumin (b) picrocroin (c) lawsone**

Betanines are natural dyes obtained from different *Beeta vulgaris* and are used as colorants in food products like yogurts, ice cream and other products (Aura et al., 2011). *Tagetes erecta* (Mexican marigold) is used as a food coloring agent and nutrient supplement in a wide range of baked goods, beverages, breakfast cereals, chewing gum and dairy products (Richard, 2004).

**Natural dyes in dye sensitized solar cells (DSSC)**

Solar energy is one of the most promising alternative energy sources for the future which replaces the limited energy resources from fossil fuels and others. The dye-sensitized solar cell (DSSC), invented by Gratzel in 1991, is a most favorable route toward sunlight harvesting which uses dye molecules adsorbed on the nano-crystalline oxide semiconductors such as TiO$_2$ to collect sunlight (Jiao et al., 2011; Silwal et al., 2020). Dye is used as a sensitizer in dye sensitized solar cells using sensitization of the cell which convert visible light into electricity. Natural dyes replacing expensive chemical synthesis process through simple extraction process by cutting down high cost of metal complex sensitizers (Verma & Gupta, 2017). The schematic representation for the principle of operation in DSSCs is shown in Figure 7.
The material choice for DSSCs is mainly TiO$_2$ (Anatase), crystal structure of which is shown in Figure 5, but alternatives such as ZnO and Nb$_2$O$_5$ have been investigated as well.

**Common health hazards caused by synthetic coloring**

There is a high risk of synthetic dyes as compared to natural dyes. Synthetic dyes or synthetic coloring is formed by using a various pigments and different metals which caused health problem and environmental pollutions (Chavan, 2013). The synthetic coloring used in textile industry contain various polluting substance as in wastewater which mixed in water and caused water pollution (Chavan, 2013; Hassaan & El Nemr, 2017). The use of synthetic coloring in food industry caused a carcinogenicity, hypersensitivity, behavioral effects, hyperactivity in children, skin problem, etc. (Okafor et al., 2016; Chavan, 2013). This results support that synthetic coloring causes various health hazardous problems.

**Economic feasibility of natural dyeing in Nepal**

There are limited numbers of so called natural dyeing small industries in Nepal but all of these are in poor economic condition on one hand and are not absolutely natural on the other (Bhandari et al., 2020). Kakani Himalayan Natural Dyes established in 2008 B.S. use to collect some wild plants and herbs mainly from jungles of Shivapuri National Park and protected areas. It was nominated for the ‘Surya Nepal Asha Social Entrepreneurship Award’ in 2012.

**Figure 7: Principle of operation in DSSC** (Gratzel, 2003)

**Figure 8: Photographs of colored fabrics with natural dyeing in Kakani Himalayan Natural Dyes Pvt. Ltd. (photo captured on industry visit in June 2019)**

To recognize the Nepali social entrepreneurs who create value for ‘People, Planet and Profit’,
is the main reason for giving this award and is given to only those social entrepreneurs who have contributed for the development of society through empowerment and job creation. Figure 8 shows the dyed fabrics kept for drying in open air inside the Kakani Himalayan Natural Dyes Pvt. Ltd. Industry at Kakani, Nuwakot, Nepal.

These are also other small industries related to natural dyeing inside and outside Kathmandu valley but are not easily available in google search. Due to the presence of varieties of natural flora and huge natural water resources, Nepal would be the better land for promoting and advancing natural coloring research based industries but it should be initiated from government level so that it can contribute career opportunities for jobless people even to remote areas to some extent.

Limitations of natural dyes

There are various advantages of natural dyes as compared to synthetic dyes, but still have some serious limitations. The major challenges working with natural dyes are the low yield in one hand and the color fixing problem on the other, although it has many compensations regarding the health issues and allergic problems. Most of natural dyeing industries use to fix or color the fabrics instantly, due to the difficulties on storing the extracted dyes for long period of time in dry conditions (Kannanmarikani et al., 2015; Das et al., 2016; Silwal et al., 2020). In many cases, it is very difficult to achieve the intended color on fabrics since it varies with water quality, water pH, mordant quantity, concentrations, time duration of coloring, and many other factors for which, a deep research and well experienced expertise are essential (Bhandari et al., 2020; Kannanmarikani et al., 2015). Further, plant based natural dyes are typically a mixture of secondary metabolites, and hence color depends on harvesting time and processing. There are also many challenges to develop secondary colors from original colors by mixing in proper quantity. The huge bio-diversity of plants in Nepal could be the research hub if all the dye yielding plants and traditional techniques could be preserved and documented properly from government level. It would be a great achievement if natural dyeing techniques by using local natural mordants such as rock salts, lemon juice, mango bark juice (Bhandari et al., 2020) could be developed. Natural dyes are expensive as compared to synthetic dyes (Khan et al., 2018) but if commercially cultivated and processed in large amount would be cheaper, safer and sustainable for the future.

RECOMMENDATIONS

Nepal is rich in biodiversity and traditional dyeing techniques and therapeutic agents. Although there are many researches related to natural dye extraction and dyeing techniques, a research based, and sustainable techniques are not in practice. In Nepal, there are so called natural dyeing industries for fabric and textile dyeing but still they use synthetic toxic mordants. So research based on natural coloring with natural mordants as the principle of green chemistry should be the prime goal. Natural color is usually mixture of compounds so very difficult to control and fix the color on fabric and textiles. To standardize various parameters such as solvents, plant ageing, extraction, purification, preservation, dyeing, pH maintain, water quality, temperature, mordants, concentrations and others, a detailed and in-depth research should have to be done. Further, research related to distribution, availability, and feasibility of growing plants could be another separate aspect to list and document the
medicinal and dye yielding plants in the country.

CONCLUSION
Nowadays, natural dyes being a sustainable and renewable bio-product with minimum environmental impacts are used as alternative to synthetic dyes. There are many parameters still has to be optimized such as green solvents for extraction, preservation, dyeing, pH effect, water quality, temperature, pre- and post-mordanting agents, concentrations and others. A renewed international interest has arisen in natural dyes due to increased awareness of the environmental and health hazards associated with the synthesis. More detailed studies and scientific investigation are needed to assess the real potential and its application on coating medicines in pharmaceutical industries, dyeing textiles and as photo sensitizer in dye-sensitized solar cells (DSSCs) from plant being carried out and will be useful for resource utilization, conservation and sustainable use of natural resources including biodiversity. The extraction and dyeing technique is not commercially succeeded like synthetic dyes due to lack of availability of precise technical knowledge. Nepal is being rich country in natural resources which exhibit the great degree of plant diversity as it possess variety of plants that can yield dye. These extracted natural dyes not only have dyeing property but also it has wide range of medicinal values.

The medicinal value thus obtain from these plants have high potential for the development of industrial production of medicines, textiles, and solar cells. Therefore, this will lead to create an employment opportunity and thus poverty alleviation to some extent. It will definitely help to document natural resources in research related with human health and green methods helps for the sustainability.

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