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Research Article

Pharmacognostic and phytochemical investigations of Royal hair dye henna available in the Libyan market

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Abstract

Background: *Henna adulteration is getting rise, over the world, as the cosmetic desire increased. Hence, health problems are induced due to its adulteration with synthetic substances. This study aimed to perform pharmacognostic and phytochemicals analysis of Royal henna to determine its anatomical features.* ***Method:*** *For this purpose, Royal (different colors) and Lawsonia inermis leaves, a natural henna herb, were purchased from local market, identified and extraction was performed by maceration of dried materials in methanol. Phytochemical screening, microscopic, thin-layer chromatographic (TLC) and florescence analyses were executed.* ***Results:*** *Microscopic studies showed presence of small parts related to senna plant within the Royal red henna extract. The Royal brown revealed presence of natural henna plant coupled with a strange plant carrying dyes unrelated to the flora of henna. While, unknown black particles and fibrous were seen in Royal black henna extract. TLC analysis revealed presence of synthetic materials. All Royal dye showed presence of foreign substances. Phytochemical screening showed presence of different primary and secondary metabolites. Whereas, the florescence evaluation revealed different colors both in visible and ultra violet (254nm and 366nm). So identification and quantification studies for foreign substances are important and needed to avoid consumer health risks.*

Keywords: Pharmacognostic, Henna, hair Dye, TLC

Introduction

As far as in recent years natural herbal products are becoming more popular worldwide. Uses of natural products, by human, are increasing due to their psychological feeling that natural products are almostally safe (Eisenberg *et al.*, 1998). However, indeed, many problems are related to herb phytochemical or its adulteration by synthetic substance (Davies and Grabczynska, 2007). A number of studies have reported certain problems that face people health issue to the risk of adverse effects (Haller and Benowitz, 2000, Ernst, 2002, Ernst and Pittler, 2002, Hung *et al.*, 2011) and toxicity (But *et al.*, 1996, Pittler and Ernst, 2003). Natural products, in particular plant origin, are widely used for their medicinal properties and cosmetic purposes. The herbal plant henna *Lawsonia inermis L., belongs to the family* Lythraceae, *is* a well-known natural dye possess both therapeutic and cosmetic benefits. In nature, there are three types of henna like neutral henna, red henna and black henna. In reality, it is used as hair growth stimulators for the treatment of dandruff and as hair colorant. Literature data demonstrates that henna plant played an important role in life of ancient and modern cultures, particularly; in the Asia and North Africa area (Pittler and Ernst, 2003). Henna is cultivated for its pharmacological actions such as antifungal, antimicrobial (Al-Rubiay *et al.*, 2008, Ahmadian and Fakhree, 2009) anti-inflammatory, analgesic, and antipyretic activities (Ali *et al.*, 1995). In addition, henna is believed as the most common natural dye for tattooing dye and hair pigmentation in many traditional cultures (Basas, 2007). Accordingly, Libyan markets are rich with different brands of henna in different preparations exported from different areas over the world. Unfortunately, the majority of these products are without quality control. Royal hair dye henna is one the genus; widely used throughout the country. Royal henna is traditionally used for a variety of ailments

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including; as a cosmetic, hair dying and treatment of wounds. So, there are possibilities that henna dye samples may get adulterated with other plant parts of different origin having similar morphology. So, it becomes very important to make an effort towards standardization of the material to be human used. So, in this scientific portion handling of standardization can be succeeded by stepwise pharmacognostic studies. It has been indicated that complete pharmacognostic studies give important information regarding the morphological, histological characteristics, quality and purity of the crude plant (Arora and Sharma, 2012). Hence, considering the importance of this product, henna, the objective of the present study was to evaluate the microscopical and phytochemical constituents of Royal hair dye henna, commercially available in the Libyan local market for public use, in order to better know its principle components.

Materials and Method

Collection of Royal hair dye henna

Three different colors of Royal hair dye henna (red, brown and black) were collected from the local Libyan market, Tripoli city, the capital of Libya. Henna authentic plant *Lawsonia inermis L.* was used in this study as a reference after it was authenticated by the taxonomy unit of the department of botany, faculty of Science, Tripoli University.

Microscopical studies

Each sample of Royal henna was subjected to a cold maceration with methanol as described previously (Deore *et al.*, 2015). Briefly, 50 mg of Royal hair dye henna or *L. inermis* were mixed separately in a separated conical flask with 100 ml of methanol. Each flask was stocked at room temperature for 48 hr. All the extracts were filtered and poured in porcelain dish and left to dry under the hood.

Thin layer chromatography

Few microliters of the methanol extract of Royal hair dye henna and *L. inermis* were spotted on thin-layer chromatographic (TLC) plates, composed of Silica gel 60 GF 254. After, the plates were developed in the chamber previously saturated by the mobile phase. The mobile phase was hexane/ ethyl acetate (90: 10 v/v ml). After drying, the plates were examined both visually and under UV light at wave length 254 and 366 nm.

Preliminary phytochemical screening

The preliminary phytochemical screening was carried out to investigate the constituent of the Royal hair dye henna and compared to authentic *L. inermis herb.* The methanol extract of all dyes were subjected to qualitative phytochemical analysis to test for the presence or absence of various active constituents such as glycosides, flavonoids, anthraquinones, alkaloids, tannins, and phenols (Khan *et al.*, 2011, Sarma and Babu, 2011).

Results and discussion

In fact, studies of natural products, of plants origin, are of great interest since plants did show useful and noxious functions in humans' life. Therefore, the pharmacognostic and phytochemical evaluation of henna plant is very important for the detection of adulterer materials and for monitoring the quality of product. *L. inermis* leaves (Lythraceae) are with wide use all over the world especially in North Africa and Asia. In the same time, different types of synthetic henna, extracted and formulated from natural sources; and are without quality control, are available

abundantly for public use. Therefore, pharmacognostic evaluations of these materials are of great interest. The pharmacognostic analysis for Royal henna hair dyes were carried out for the first time in this study. Our observation revealed that there is a great difference in microscopical, phytochemical and TLC profiles between the Royal hair dye henna, a man-made type of henna, and the authentic plant *L. inermis*. Microscopic examination of *L. inermis* leaves powder revealed presence of parenchymatous cell, rosette crystal of calcium oxalate and fibers. The epidermal layer was distinguished with stomata (fig. 1, I). The Royal hair dye powder when examined under microscope display the following inclusions. Royal brown color hair dye henna revealed presence of several cluster of calcium oxalate crystal, presence of parenchyma tissue and fibers (fig. 1, II). Anomocytic stomata were also seen. Royal red color hair dye henna is characterized by presence of prismatic calcium oxalate sheath and unicellular warty hairs. The stomata were dicytic type (fig. 1, III). Dark unidentified particles were only observed in Royal black color hair dye henna (fig. 1, IV). Though, senna is not mentioned in the Royal henna package, senna features were seen under microscope as diacytic stomata and unicellular warty hairs trichomes (fig. 3). This observation was expected since, in Asian countries, senna is known to be found in natural henna as a part of their natural dyes (Ali *et al.*, 1995). The observation of dark particles gives an indication for the presence of foreign substance as paraphenyldiamine (PPD). Indeed, PPD is added to many henna formulations to darken its color and hence, PPD is considered as a major constituent of permanent hair dyes (Basas, 2007). In actual fact, PPD adverse effects and toxicity such as dermatitis and renal failure were previously reported in details (Arroyo, 2003, Davies and Grabczynska, 2007, Jacob *et al.*, 2008).

The phytochemical screening of hair dyes demonstrated a similarity between the authentic *L. inermis* leafs and Royal red color henna, but not to brown and black colors henna. The methanolic extract was found to contain glycosides, phenolic compounds, tannins, flavonoids and anthraquinone; while alkaloids was not found in both authentic *L. inermis* and Royal red color hair dye henna, as shown in Table 1. The methanol extract obtained from Royal brown hair dye was tested positively for tannins, flavonoids, and anthraquinone. While, Royal black hairs dye henna revealed presence only of flavonoids and anthraquinone compounds. Fluorescence analyses of henna powder were examined both, in the visible day light and under UV light at 254 nm and 366 nm (fig. 2). TLC chromatograms developed using hexane: ethyl acetate (90:10) as a solvent system. Royal red and brown colors henna showed a distinct yellow fluorescence bands under long wave UV light at 366nm indicating the possibility of the presence of identical compounds. Hence, the fluorescence character of the dye powders helps in qualitative evaluation which can be used as a reference data for the identification of adulterations.

Conclusion

Royal hair dye henna, available in the Libyan local market, contains different primary and secondary metabolites. The methanol extract tested positively for glycosides, phenols, tannins, flavonoids and anthraquinone. The present pharmacognostic

studies revealed that Royal henna dyes, as a pharmaceutical product, are not pure and contains, in addition to other different natural plant parts, fake materials. So, various standardization parameters such as microscopy, TLC and phytochemical screening are needed to be carried out since it could be helpful in authentications of adulterated type of hair dye henna. The results of this study provide valuable information and can be serving as a reference material in the preparation of plant monograph. Also, more studies are needed in order to confirm and taking into account the quality and purity of the dye material.

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Fund. None

Table 1: Phytochemical elements found in authentic plant L. inermis and Royal hair dye henna. (+) indicates present; (-) indicates absent

Substance	*L. inermis leaves*	Royal hair dye henna		
		Black	Brown	Red
Alkaloids	-	-	-	-
Glycosides	+	-	-	+
Phenols	+	-	-	+
Tannins	+	-	+	+
Flavonoids	+	+	+	+
Anthraquinone	+	+	+	+

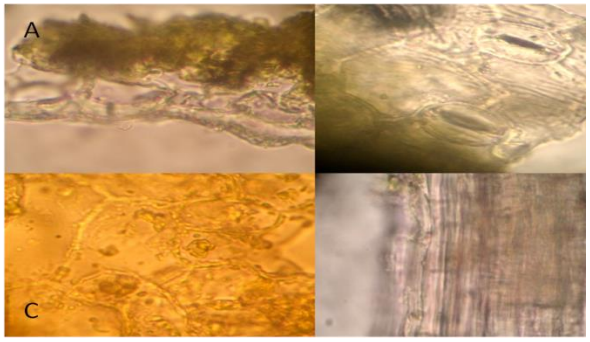
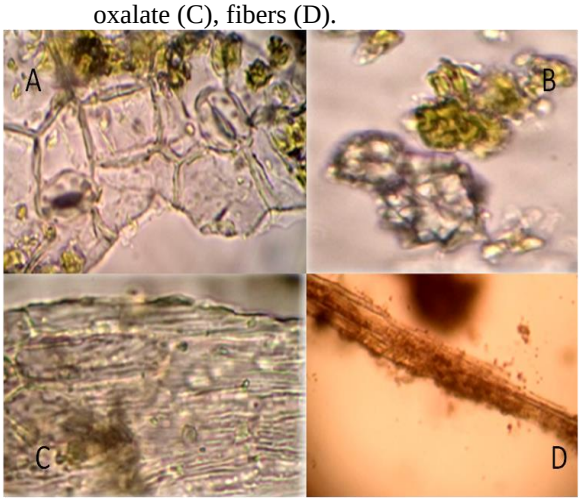
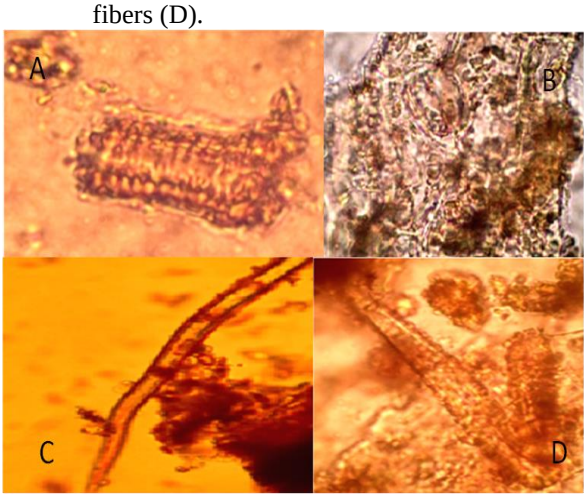


Figure 1:

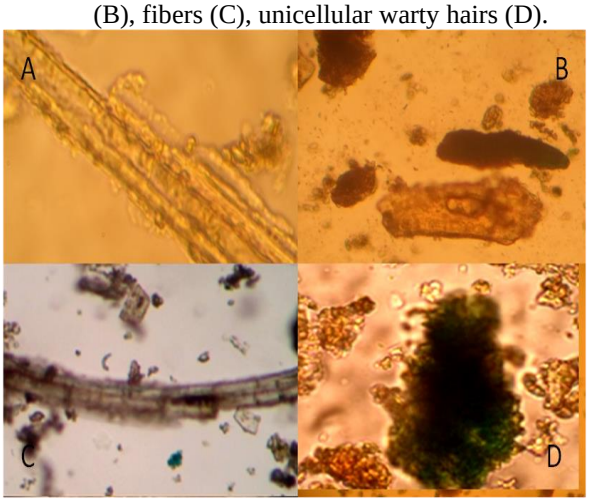
I. Authentic L. inermis leafs; fragment of parenchymatous cell (A), epidermal layer with stomata (B), rosette crystal of calcium oxalate (C), fibers (D).



II. Royal brown color hair dye henna; anomocytic stomata (A), cluster of calcium oxalate crystal (B), parenchyma tissue (C), fibers (D).



III. Royal red color hair dye henna; prismatic calcium oxalate sheath (A), diacytic stomata (B), fibers (C), unicellular warty hairs (D).



IV. Royal black color hair dye henna; fibers (A) unidentified dark particles (B, C, D).

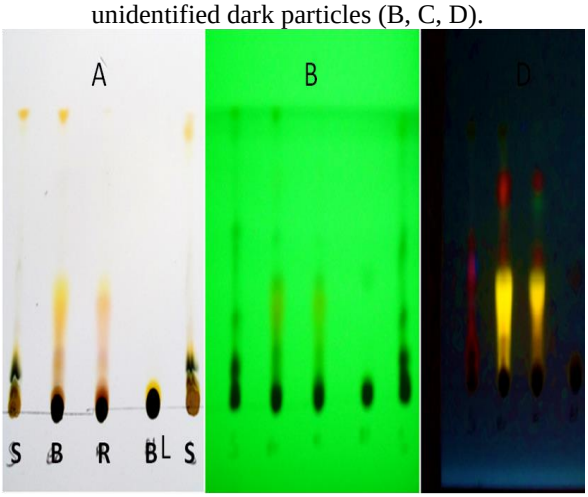


Figure 2: Thin layer chromatographic profile of authentic L. inermis (S) and Royal hair dye henna (B= Brown, R = Red, BL = Black). Detection of bands was done by visual (A), UV light at 254 nm (B) and at 366 nm (C).

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