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Evaluation of Ubtan – A traditional indian skin care formulation

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ABSTRACT

Ethnopharmacological relevance: 'Ubtan' is a traditional herbal formulation in the Indian system of medicine being used in India and its subcontinent for a long time. Several commercial skin care formulations are marketed throughout this region as the name of Ubtan. Therefore, it is worthwhile to evaluate Ubtan in respect of its efficacy as skin care formulation.

Aim of the study: The present study was designed for the preparation of Ubtan and standardization through the chromatographic techniques by using suitable phyto-markers. Further, its antioxidant, sun protection factor (SPF) and anti-tyrosinase potential have been explored.

Materials and methods: Four in-house formulations (UF-1, UF-2, UF-3 and UF-4) were prepared by mixing a varied quantity of each powdered plants, i.e. turmeric (*Curcuma longa* L.), Chickpea (*Cicer arietinum* L.) and sandalwood (*Santalum album* L.). Optimization of the formulations was made by evaluating its biological activity through in vitro assay. Evaluation of physicochemical properties of the optimized formulation (UF-1) has been carried out by analysis of pH, flow properties and stability. Moreover, RP-HPLC (reverse phase - high performance liquid chromatography) and HPTLC (high performance thin layer chromatography) standardization of UF-1 was performed for its quantitative and qualitative analysis.

Results: Ubtan formulations (UF-1to UF-4) showed free radical scavenging and ferric reducing potential. It may be due to its high phenolic and flavonoid content. Statistically, significant Pearson's correlation (r) was confirmed the positive correlation between phenolic content and SPF of the formulations. The tyrosinase inhibition study indicated that the formulations showed both diphenolase and monophenolase inhibitory activity. Among four formulations, UF-1 showed notable biological activity ($p < 0.05$). The content of curcumin and ascorbic acid was found to be 1.6% and 2.1% w/w respectively in UF-1 through RP-HPLC estimation. Physiochemical properties of the UF-1 exhibited good flow rate and aqueous solubility. From the stability studies, it can be *anti*cipated that the UF-1 was stable at 40 °C for longer periods. Microbial load count and heavy metal content (lead-Pb, arsenic-As, mercury-Hg and cadmium-Cd) of the formulation was also within the permissible limit of a pharmacopeial standard.

Conclusion: This scientific exploration helps to set the quality and safety standard of traditional cosmetic formulation, Ubtan and its further use as an herbal skin care product.

1. Introduction

'Ubtan' is a semisolid preparation of powdered formulation used to remove the dirt particles from the skin and enhances the luster of the body. Ample of evidence suggested that *Curcuma longa* present in Ubtan to enhance the skin colour (Dixit and Goyal, 2011). The ritual 'Solah Shringar' encompasses sixteen ways of beautifying of a woman's body in the Hindu as well as Muslim marriages in India. Ubtan paste is one of the vital components of Solah Shringar (Miczak, 2001). It is also used in Ayurvedic body massage known as 'Ubvartan' which makes skin soothen and soft (Sachs, 2002). Ubtan is prepared by mixing of *C. longa* (turmeric) and *Cicer arietinum* (Chickpea) along with other ingredients in rational quantity (Jamal et al., 2005). It is used as cleanser, skin tonic and refresher among the local communities of Pakistan (Mushtaq et al., 2012). Women in South Asia believed that it lightens the complexion of the skin, making the baby more beautiful (Reissland and Burghart, 1987). Ubtan, is a herbal

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cosmetic formulation, being used traditionally in India and other Asian countries. It's manufacturing, sales and distribution in India is governed by drug and cosmetic act 1945 (Javed et al., 2009; Lohar, 2007a). However, none of the official standards have been developed to maintain its quality and safety concern.

Polyphenol oxidase or tyrosinase (EC 1.14.18.1) is a multifunctional copper containing an enzyme, responsible for the synthesis of melanin within the melanocytes. Two major actions of this enzyme are the hydroxylation of L-tyrosine to L-DOPA (monophenolase) and subsequent oxidation of L-DOPA to dopaquinone (diphenolase). Dopaquinone is utilized further in intermediated biosynthetic steps to produce melanin. It's a mixture of heterogeneous biopolymers regulating the skin colour. It plays a protective role by absorbing ultraviolet (UV) sunlight and removing reactive oxygen species (ROS) from skin (Biswas et al., 2015a). Accumulation of an excess amount of melanin in the skin may result in hyperpigmentation. Melasma is the most common hypermelanosis disorder in the Asian region among people who live within the area of intense solar exposure. Thus, tyrosinase inhibitors are clinically useful therapeutic agents for melanin associated darkening of the skin (Pasricha et al., 2007). However, controlling for hyperpigmentation disorders is challenging due to the limited number of currently available treatments.

Some organic and inorganic mercury salts are widely used in skin whitening products due to its melanin inhibitor perspective. These are absorbed through the skin and produce harmful side-effects in the body, e.g. kidney damage, skin discoloration, allergic reaction and scarring, as well as the skin, loses its resistance to bacterial and fungal infections (Al-Saleh et al., 2004). In India, 61% of the dermatology market consists of skin lightening products. Several tests have been carried out to analyze the heavy metals like As, Hg, Cd, Pb and trace elements in herbal cosmetic's preparations sold in the Indian market (Ladizinski et al., 2011). Some of them contain heavy metals above the safety limits.

Ubtan is one of the popular beautifying cosmeceutical in India developing from traditional knowledge. The scientific standard of Ubtan was developed based on its phytochemical and therapeutic claim. The present study was designed for the preparation of Ubtan and its standardization through RP-HPLC techniques by using suitable phyto-marker. Further, it was explored for its anti-oxidant and anti-tyrosinase activity. In addition, heavy metals were estimated by the atomic absorption spectroscopy (AAS) for its assessment of safety for topical application.

2. Material and method

2.1. Chemicals and reagents

All solvents and reagents (methanol, acetonitrile and glacial acetic acid) used for chromatographic analysis were of HPLC grade that was procured from Merck (Darmstadt, Germany). All other reagents and chemicals used in this study were of analytical grade. Kojic acid (Product ID: K3125-5G), mushroom tyrosinase (Product ID: T3824-25KU) 1-Diphenyl 2-picrylhydrazyl, quercetin, butylated hydroxyanisole (BHA), ascorbic acid, curcumin, Sandalwood oil from *Santalum album* L. (84,475–5 ml), L-tyrosine and L-DOPA (Levodopa) were purchased from Sigma-Aldrich, Inc (St. Louis, MO, USA).

2.2. Plant material collection, authentication and physicochemical evaluation

The rhizome of *Curcuma longa* L. (family - Zingiberaceae), seeds of *Cicer arietinum* L. (family - Fabaceae) and heartwood of *Santalum album* L. (family - Santalaceae) were purchased from an authorized Ayurvedic herb vendor, Jadavpur, Kolkata, India in the month of October 2012. Plant specimens were identified and authenticated by Dr. S. Rajan, Field botanist, the medicinal plant collection unit, Ooty, Tamilnadu, Govt. of India. The voucher specimen numbers of the plant specimens used in the formulation was SNPS-JU/2012/1106 for *C. longa*; SNPS-JU/2012/1107 for *C. arietinum* and SNPS-JU/2012/1108 for *S. album*. Specimens were preserved in the School of Natural Product Studies, Jadavpur University, Kolkata, India for future reference. The collected plant materials were shade dried. The crude plant materials were subjected to standardization according to the WHO guidelines, e.g. organoleptic properties, physiochemical characteristic, heavy metal estimation and preliminary phytochemical analysis (WHO, 2011).

2.3. Development of Ubtan formulation

The formulation was prepared as suggested by the registered Unani medicine practitioners. Three primary ingredients selected for Ubtan formulation, i.e. rhizomes of *C. longa* (turmeric), seeds of *C. arietinum* (chickpea) and heartwood of *S. album* (sandalwood). Further, pulverized plant materials were made into fine powder by passing through a sieve (No. 80). Accurately weighed turmeric, chickpea and sandalwood powders were mixed in the ratio of 2:2:2 w/w/w (UF-1), 1:2:2 w/w/w (UF-2), 2:1:2 w/w/w (UF-3) and 2:2:1 w/w/w (UF-4), respectively. Three powders were added one by one to mix thoroughly in a plastic tray. It was triturated uniformly to make a homogeneous mixture. After mixing formulation was transferred into a plastic container and stored in a cool and dry place for further study. Four different formulations have been developed randomly to validate their traditional claims scientifically.

2.4. Determination of total phenolic and flavonoid content

The total phenolic content (TPC) was analyzed according to the Folin-Ciocalteu method (FCM) (Siddhuraju and Becker, 2003; Bray and Thorpe, 1954). 200 µl of aqueous extract of four in-house formulations were taken into individual test tubes, and the volume was made up to 2 ml with distilled water. 1 ml of Folin-Ciocalteu phenol reagent (1:1 with water) and 5 ml of sodium carbonate solution (20%) was added. After that, the reaction mixtures were placed in dark for 40 min, and the absorbance was recorded at 725 nm against the blank by using Spectrophotometer. The content of total phenolic compounds was calculated as milligram of gallic acid equivalents (GAE) in 1 g of the sample. The result was represented as mean ± standard deviation (SD) (n=3).

The total flavonoids (TF) were calculated by a spectrophotometric assay (Zhishen et al., 1999; Woisky and Salatino, 2015). 1 ml of standard solutions of quercetin or test samples was prepared at different concentrations to range (1.5–100 µg/ml). The volume was made up to 4 ml by adding distilled water. At the beginning of the experiment, 0.5 ml of 3% $NaNO_2$, 3 ml of 15% $AlCl_3$ and 2.5 ml of 1 M NaOH were added to make a total volume of 10 ml and mixed thoroughly. The absorbance of the mixture was determined at 415 nm Vs the blank. The content of total flavonoids was calculated as milligram of quercetin equivalents in 1 g of sample. The result was represented as mean ± standard deviation (SD) (n=3).

2.5. Anti-oxidant activity of Ubtan

2.5.1. Diphenyl-1-picryl hydrazyl (DPPH) assay

100 µl of sample solution of different concentration was mixed with 150 µl of DPPH solution (0.20 mg/ml) in 96-well micro-plate (VersaMax™ ELISA Microplate). The mixture was incubated for 20 min at room temperature in the dark. The absorbance of the