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# Embelin-A natural potential cosmetic agent

**N. Radhakrishnan, V. Kavitha, STK. Raja, A. Gnanamani, A.B. Mandal**
Microbiology Division Central Leather Research Institute (CSIR, NewDelhi) Adyar, Chennai, Tamil Nadu, India

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## Summary

*Embelia ribes* one of the Indian traditional medicinal plant, has been used as a cosmetic agent to cure skin disorders for centuries. *E.ribes* is used especially for dyeing hairs, good pimple remover, treating acne, treating carbuncle infections, treating vitiligo and leucoderma. *E. ribes* berries contain a quinone derivative embelin (2,5-dihydroxy -3-undecyl,1,4- benzoquinone), has a wide spectrum of biological activities, such as antioxidant, antitumor, anti-inflammatory and analgesic, antihelmintic, antifertility and antimicrobial. Quinone derivatives and the analogs; Ubiquinone (Coenzyme $Q_{10}$), Idebenone, Arbutin and Hydroquinone are well-known for cosmetic applications. In the present study, embelin from *E.ribes* berries of Indian origin was extracted and characterized by UV and FT-IR analyses.

Hemolytic, tyrosinase and DOPA auto-oxidation assays were also carried out. About 1.9± 0.1 gram of pure embelin was obtained from 100 gram of powdered berries *(E.ribes)*. The characteristics studies reveal the properties are on par with the standard embelin received from Sigma (USA). The half-maximal effective concentration ($ED_{50}$) of embelin to cause hemolysis was found as 109± 0.1 μg/ml. The tyrosinase inhibitory activity of embelin was nil and the DOPA auto-oxidation activity was observed up to 350 μg /ml concentration. Thus the embelin finds, potentially application in cosmetic industries.

## Riassunto

*L'Embelia ribes*, una delle piante usate nella medicina tradizionale indiana, è stata utilizzata per secoli come agente cosmetico per curare alcune forme patologiche.

*E. ribes* è usata soprattutto per colorare i capelli, per eliminare le piccole pustole acneiche e per trattare la vitiligine e le leucodermie.

*E. ribes* contiene un derivato chinonico (2,5-diidrossi-3-undecil, 1, 4-benzochinone) che possiede un ampio spettro di attività biologiche

agendo come antiossidante, antitumorale, antiinfiammatorio, analgesico, antielmintico, antifertilità e antimicrobico.
Alcuni derivati chinonici quali l'ubichinone (coenzima $Q_{10}$) l'idebenone, l'arbutina e l'idrochinone sono ben conosciuti per il loro uso cosmetico.
Con il presente studio è stata estratta l'embelina dalla E. ribes caratterizzandola attraverso l'uso dell'UV, FT e IR. Ne è stata anche valutata l'attività emolitica e l'attività sulla tirosinasi e sulla catena DOPA.
Da 100 gr. di *E. ribes* in polvere è stato ottenuto circa 1.9 ± 0.1 gr. di embelina pura, che ha rivelato di possedere le stesse caratteristiche dell'analogo prodotto ricevuto dalla SIGMA (USA).
La concentrazione massima di embelina che provoca emolisi al 50% $8ED_{50}$) è di 109 ± 0.1 μg./ml.
L'attività inibitoria nei confronti della tirosinasi è risultata nulla, mentre l'attività autossidante nei confronti della DOPA è stata riscontrata pari a 350 μg/ml.
Per tali caratteristiche, l'embelina può trovare potenziali applicazioni d'uso nel comparto cosmetico.

## INTRODUCTION

Beauty, the quality that gives pleasure to the senses, is perhaps the desire of every human being on earth especially in Asian. Some are born beautiful and some are in fact made beautiful. Aesthetic appearance has always been a matter of main importance. The word 'beauty' is not only related to women gender, as is often thought, but men also intend to use cosmetic products as daily basics. The knowledge of herbal cosmetics is booming now-a-days by both verbal transmitted folk information and newer information generated by modern scientific studies. Herbal products like extracts, oils and powders have been used in cosmetics as either active moieties or as excipients (as individually or in combination). Herbal extracts are primarily added as cosmetic ingredients due to several value added properties such as antioxidant, anti-inflammatory, anti-aging and anti-tyrosinase etc. Kole et al. (14) reported some Indian medicinal plants, which have been used in cosmetic preparations. And he added that *Embelia ribes* one among the plant has been used as a cosmetic agent to cure skin disorders for centuries. Samatha and Vasudevan (30) reported that aqueous extract of *E.ribes* on acid hydrolysis yield a colored product, which is used for dyeing cotton, nylon, silk and wool. And added *E.ribes* can be used as substitute for the synthetic red colors.

Samatha and Vasudevan (31) also reported embelic acid isolated from *E.ribes* for dyeing hair. However retention of the color is inferior to *Pterocarpus santalinus* extract studied. The uses of *E.ribes* have been mentioned in Ayurvedic texts. Susrut recommended *E.ribes* rasayana for longevity (anti-aging), while Vagbhata recommended *E.ribes* in treating leprosy, as an anthelmintic in ringworm and bulbous eruptions (33). *E.ribes* is one of active ingredient of TARIKA herbal pimple remover, product of Ayurlabs India, they conducted a clinical trial studies for the same and observed as good herbal pimple remover in all cases of acne patients (20).

Paranjpe and Kulkarni (21) reported one of Ayurvedic formulation namely; Sunder Vati showed a significant improvement in inflammatory and non-inflammatory lesions compared with baseline or placebo in Indian patients with *Acne vulgaris*. Anand Kumar and Sachidanand (2) conducted a clinical trial studies with new polyherbal formulations CLARINA cream and PURIM tablets. *E.ribes* is one of ingredients of CLARINA cream and thus reported that CLARINA cream along with PURIM tablets was useful in treating patients with various degrees of acne. Saikia et al. (28) reported *E.ribes* along with *Emblica officinalis, Piper longum and Terminalia belerica* all the four herbs are mixed equally and the crushed form dissolved in honey and applied for treating carbuncle infections. "PIGMENTO", an ayurvedic tablet marketed by Herbal Ayurvedic Remedies (www.herbal ayurvedic remedies.com) also contains *E. ribes* as one of ingredients, which is used for treating vitiligo and leucoderma.

The Government of India recently has set up a National Medicinal Plants Board (under the Ministry of Indian System of Medicine and Homeopathy) for over all development of medicinal plants and its knowledge. The Board has identified 32 prioritized medicinal plants, *E.ribes* is one of plant which has gained national importance owing to its therapeutically and commercial need especially of *E.ribes* berries (29). *E.ribes* is also one among the top 20 ayurvedic drugs of India as reported by Patwardhan et al. (23).

*E. ribes* berries contain a quinone derivative embelin, an alkaloid christembine, a volatile oil and vilangin. (26). Among them, embelin is considered one of the major bioactive constituents and marker compounds in *E. ribes* berries (4; 5; 9). Embelin (2, 5-dihydroxy -3-undecyl, 1, 4-

benzoquinone) has a wide spectrum of biological activities, including antioxidant (13), antitumor (10), anti-inflammatory & analgesic (8), antihelmintic, antifertility (15) and antimicrobial (9).

In the present study, we have extracted embelin from *E.ribes* (Indian origin) and examined the hemolytic potential of embelin (2, 5-dihydroxy-3-undecyl-2, 5-cyclohexadiene-1, 4-benzoquinone) in human erythrocytes. And also investigated the mushroom tyrosinase activity and DOPA autooxidation potential of embelin.

# MATERIALS AND METHODS

## Plant material

*E.ribes* berries were obtained from M/s Abirami Botanical Corporation, (Tuticorin, TamilNadu, India) and it was authenticated by Dr. Anandan, Research Officer, Anna Hospital, Chennai.

## Extraction of the Embelin

Extraction of embelin was carried out according to Indian herbal pharmacopoeia (3). 100 g of the powdered berries of *E.ribes* was extracted with n-hexane using a soxhlet extractor for 6 hrs. The extract was then evaporated on rotator vapor and recrystalized using ethanol and chloroform and characterized using UV Visible and FT-IR analyses according to the standard methods.

UV-Visible spectrum was recorded using UV-2450, Shimadzu (Japan) in the wavelength range between 190-800 nm. FT-IR spectral measurements was made using Spectrum one (Perkin-Elmer Co., USA model).

## Hemolytic activity

The hemolytic activity of plant materials or its preparation was determined using suspension of erythrocytes cells (RBC), mixed with equal volumes of a serial dilution of the plant material according to WHO guidelines (34).

Blood sample was aseptically collected in hepairnized sterile tube from healthy volunteer, after getting their consent for experiment. Blood samples were centrifuged at 10,000 rpm for 20 minutes at 4°C, to remove the cell debris. Resultant pellet was washed (3-4 times) repeatedly with phosphate buffer saline (PBS) pH 7.4 to obtain erythrocytes cells (RBC). This resultant erythrocyte cells (RBC) was suspended in phosphate buffer saline containing test solution at concentration of 20-125 µg /ml and was incubated at room temperature for 10 minutes in the dark. At end of incubation, tubes were centrifuged at 6000 rpm for 20 minutes at 4°C, in order to separate the intact cell and debris.

The amount of released hemoglobin (Hb) in the supernatant was measured spectrophotometrically at 540 nm. The half-maximal effective concentration ($ED_{50}$) of hemolysis was then calculated from the resulting dose response curve.

## Tyrosinase activity

Tyrosinase activity was determined by Dopachrome method using L-tyrosine and mushroom tyrosinase as substrate and enzyme source respectively as reported earlier by Radhakrishnan et al. (25). In brief reaction mixture of control tube constitutes 235 µl of 3 mM L-tyrosine, 285 µl of 50 mM phosphate buffer (pH 6.8) and 180 µl of mushroom tyrosinase. In the case of experimental samples, buffer and test solution was adjusted accordingly. The assay mixture was incubated at 37°C, 10 minutes prior to enzyme addition and 20 minutes after enzyme addition. The pink color formed (Dopachrome) was measured spectrophotometrically at 475 nm. Kojic acid was used as reference.

### DOPA auto-oxidation assay

DOPA auto-oxidation was determined by Dopachrome method using L-DOPA and Riboflavin as substrate and source of producing oxygen radical respectively as reported earlier by Radhakrishnan et al. (25). In brief reaction mixture of control tube constitutes 250 µl of 4 mM DOPA, 200 µl of 26 µM Riboflavin and 550 µl of 50 mM phosphate buffer (pH 7.5). In the case of experimental samples, buffer and test solution was adjusted accordingly. The assay mixture was irradiated under fluorescent lamp for 15 minutes. The pink color formed (Dopachrome) was measured spectrophotometrically at 475 nm. Kojic acid was used as reference.

## RESULTS

About 1.9± 0.1 gram yield of pure embelin (figure.1) was obtained from 100 gram of powdered berries (*E. ribes*). In the UV spectrum, embelin exhibited λmax at 289 nm as shown in the figure.2; similarly IR spectrum exhibited various characteristics functional groups as shown in the table.1. Other instrumental analyses, viz., NMR, DSC, TGA and XRD, were carried out and the obtained results are compared with the standard embelin obtained from Sigma, USA (results are not shown) and found the characteristic features are on par with the standard embelin.The half-maximal effective concentration ($ED_{50}$) of embelin to cause hemolytic was found as 109± 0.1 mg/ml. Embelin does not have any action on reducing or inhibiting the Mushroom tyrosinase activity even at 125µg /ml, whereas the kojic acid (reference compound) showed half-maximal inhibitory concentration ($IC_{50}$) as 1.9± 0.1 µg /ml. Similarly the chosen test compound does not have any action on reducing or inhibiting the DOPA auto-oxidation reaction even at 350 µg /ml, whereas reference compound (kojic acid) showed half-maximal inhibitory concentration ($IC_{50}$) as 197± 0.2 µg /ml.

## DISCUSSION

In the present study extraction of embelin from 100 gm of *E.ribes* using hexane provided 1.9± 0.1 gram of pure embelin, while Chitra et al. (9) reported 0.3 g yield of embelin. However, Chauhan et al. (5) and Madhavan et al. (18) reported 4.8 g and 3.8 g yield of embelin respectively. Followed by extraction the characterization studied of embelin revealed identical UV spectra peak similar to the results of Madhavan et al (18), Shelar et al. (32) and Babu Ganesan et al. (4). With regard to FT-IR analysis of embelin, the results are similar to the observation made by Madhavan et al. (18), Kumara Swamy et al. (16), Pathan & Bhandari (22) and Cherutoi et al. (6).

**TABLE I**

*FT-IR analysis of embelin extracted from E.ribes.*

| S.No | Wave number( $cm^{-1}$) | Assignment |
|---|---|---|
| 01 | 3309 | Hydroxyl group |
| 02 | 2923 | Alkane group |
| 03 | 2854 | Methylene group |
| 04 | 1612 | Carbonyl group |
| 05 | 1326 | Aromatic C-O streching |
| 06 | 771 | Benzene group |

With reference to toxicity of embelin, Chen & Chen (7) reported that overdose of embelin can lead to renal toxicity. Pichaya and Warinthorn (24) reported embelin, showed high cytotoxicity against brine shrimp at $LC_{50}$ 1.72 µg /ml, while Alluvri et al. (1) reported *E.ribes* berries, showed lethality against brine shrimp at $LC_{50}$ 463 µg /ml after 24 hrs exposure. Haq (12) reported *E.ribes* causes visual defects leading to optic atrophy. The eye irritation and primary skin irritation studies of embelic acid conducted by Samatha & Vasudevan (31) revealed that embelic acid is non-irritant. In addition, no reports are available on the hemolytic activity of embelin. In the present study $ED_{50}$ of embelin to cause hemolysis was found as 109± 0.1 mg/ml. However, Kuznetsova et al. (17) reported hemolytic activity for 2, 3-dimethoxy-5-methyl-6-polyprenyl-1, 4-benzoquinone (Ubiquinone $Q_9$) with concentration range of 30-50 µg /ml in mouse erythrocytes.

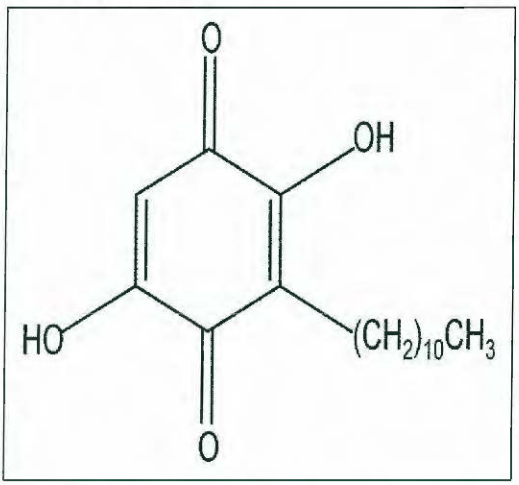


***Fig. 1*** *Represents the chemical structure of embelin (2, 5-dihydroxy -3-undecyl, 1, 4- benzoquinone).*

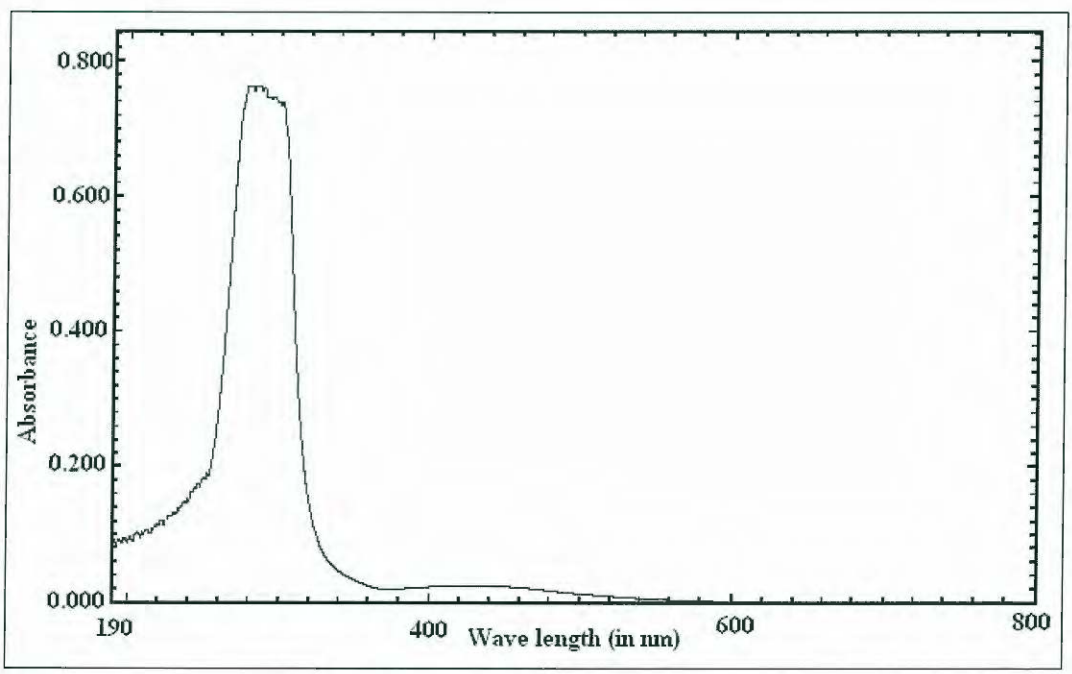


***Fig. 2*** *Represents the UV-Visible spectrum of embelin extracted from E.ribes.*

Zinkham and Oski (35) reported henna *(Lawsonia alba)* a cosmetic agent as potential cause of oxidative hemolytic and neonatal hyperbilirubinemia. While Raupp et al. (27) also reported henna (L.alba) causes life threatening hemolytic particularly in individuals with a genetic deficiency in erythrocytic glucose-6-phosphate dehydrogenase activity. McMillan et al. (19) observed no hemolytic activity for lawsone (2-hydroxy-1, 4-naphthoquinone) an active ingredient of henna *(L.alba)* and also for THN (1, 2, 4-Trihydroxynaphthalene) even at high concentration (>3 mM). Ediriweera et al. (11) reported *E.ribes* as one of the ingredient of Kaishor guggulu ayurvedic preparation used for treating shvitra (Vitiligo), similarly results of present study emphasize, embelin does not inhibit the tyrosinase activity and DOPA auto-oxidation activity and suggests its potential use as a cosmetic agent.

## CONCLUSION

Thus the embelin finds, potentially application in pharmaceutical and cosmetic industries.

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## Author Address:

Dr. A. Gnanamani
Microbiology Division - Central Leather Research Institute
Adyar, Chennai 600020 - Tamil Nadu, India
Email: gnanamani3@gmail.com
Fax: 91-44-249112150