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Selective Extraction of Embelin from Embelia ribes by Hydrotropes

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Selective Extraction of Embelin from Embelia ribes by Hydrotropes

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Abstract: The research work proposes an alternate strategy of the extraction of embelin (2,5-dihydroxy-3-undecyl-p-benzoquinone) from *Embelia ribes*. The aromatic hydrotropes such as sodium n butyl benzene sulfonate (NaNBBS), and sodium cumene sulfonate (NaCS) were found to be effective for the selective extraction of embelin with a recovery of 95% embelin from the aqueous solution of hydrotropes with high purity. The process was further optimized with respect to concentration of hydrotropes and temperature of extraction.

Keywords: Embelin, *Embelia ribes*, sodium cumene sulfonate, sodium n butyl benzene sulfonate

INTRODUCTION

Quinone constitutes one of the earliest known groups of naturally occurring organic compounds. The major attraction was their color, but the recent interest in them is due to their biological activities (1–3). Benzoquinones are the simplest representatives of quinone group. Embelin (2,5-dihydroxy-3-undecyl-p-benzoquinone) Fig. 1, is found to be the active principle of *Embelia ribes*. It is reported to posses a wide spectrum of biological activities (4–11).

Conventionally embelin is extracted from the dried berries of *E. ribes* by soxhlet extraction or percolation using organic solvents such as benzene, diethyl ether, ethyl acetate, or ethyl alcohol. The extraction, purification, and quantification of embelin are time consuming and involved multiple

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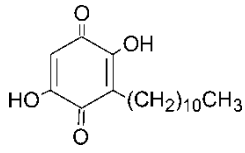

Figure 1. Structure of embelin.

extraction and purification steps (12–13). This article describes the development of an aqueous solution based extraction of embelin from *E. ribes.*

Hydrotropes are the organic salts of benzoic and salicylic acids, benzoic sulfonic acids, and its many derivatives of naphthoic and various hydroaromatic acids which when present in aqueous solution can increase the solubility of various hydrophobic organic substances in the aqueous phase. Most of the hydrotropic solutions precipitated the solutes out of the solution on dilution with water thus enable the ready recovery of the dissolved solutes (14). Hydrotropy is the phenomena of increasing solubility of sparingly soluble organic compounds in aqueous solutions in the presence of these organic salts. Hydrotropy is one of the potentially attractive techniques similar to micellar solubilization but with a much higher capacity. The amphiphillic hydrotrope molecules aggregate to form micelles. These structures are analogues to phospholipid bi-layers of cell membrane. The hydrotropic effect is significantly above minimum hydrotropic concentration (MHC) that is characteristic for a given hydrotrope. The solubility of an organic compound in the hydrotropic solution rises almost exponentially immediately above MHC; but at higher concentrations of the hydrotrope, it might level off to a plateau depending on the nature of the solute. When the hydrotropic solution drops below its MHC the dissolved solute will be recovered (15) Dilution of the saturate solution with water is sufficient to recover the dissolved solute. These phenomena can be used for the separation of close boiling point substances. The first research work of the use of hydrotropy for the separation of aniline, diethylaniline mixtures was reported by McKee.(16) High selectivity of hydrotropes in extraction was subsequently investigated by many others (17,18). Efforts are underway to exploit the ability of hydrotropes to recognize a closely related substance for preferential extraction of hydrophobic naturally occurring compounds (19–22).

EXPERIMENTAL

The aromatic sulfonate hydrotropes sodium cumene sulfonate (NaCS) and sodium para toluene sulfonate (NaPTS) and sodium xylene sulfonate (NaXS) were purchased from Navdeep Ltd., Mumbai, India and were recrystalized in MeOH. Sodium n butyl benzene sulfonate (NaNBBS) was synthesized in the laboratory by sulfonation of n-butyl benzene ring using

H_2SO_4 (98% w/v), followed by neutralization of the NaOH solution and recovered by recrystallization. The solvents used for the experiments were AR Grade solvents. The raw material *E. ribes* berries were obtained from Simosis International, India. Embelin of purity 97% (Sigma–Aldrich) was considered as the external standard for the estimation of embelin.

E. ribes berries were powdered and separated by a mechanical sieve of different sizes. Powder size of 50 μm was used as the raw material for extraction.

Two gms of the raw material was continuously extracted in a soxhlet with diethyl ether for 12 hr in order to detect the percentage of embelin content in berries. It was found that 3.1% of embelin was present in the berries used for the present experiment (23).

The extraction was carried out in a fully baffled borosilicate cylindrical glass vessel (9 cm height, 7 cm i.d.), equipped with a six bladed turbine impeller (2 cm i.d.).

The loading concentration of raw material was 5% (w/v). The raw material was suspended in 100 ml hydrotrope solution. Temperature was maintained at $28 \pm 2^{\circ}C$ for extraction studies. The extraction studies were conducted for 3 hr. The effect of temperature on the pattern of embelin extraction was studied at $40^{\circ}C$ and $50^{\circ}C$ for 3 hr. An equal amount of sample was withdrawn at a time interval of every 30 minutes. Speed of agitation was maintained at 1200 rpm. After extraction the solution was allowed to settle for 30 minutes and filtered under vacuum. The filtrate was diluted with acidified water (pH 4) in order to bring the hydrotrope concentration below MHC. Embelin precipitated from the solution as fine orange colored crystals over a period of 20 minutes. The suspension was centrifuged at 5000 rpm for 15 minutes to separate embelin from the remaining solution. The recovered embelin was washed with distilled water and dried under vacuum to remove moisture. The embelin was analyzed by a high performance thin layer chromatography (HPTLC) equipped with the sample applicator Linomat-IV, TLC Scanner III and integration software, CATS 4.05. The analysis was performed in 20×20 cm silica gel 60 F_{254} plates from E. Merck Germany. The plates were developed in a solvent system ethyl acetate:formic acid:acetic acid:water (94 : 1 : 1 : 2) in a TLC chamber. The wavelength for scanning was 280 nm. For quantification a standard curve was plotted using standard embelin as the external standard.

After the recovery of embelin the aqueous hydtrotrope extracts were measured and dried. The total inorganic phosphorous content in the extract was estimated using sodium molybdate and hydrazine sulphate at 650 nm (24).

RESULTS AND DISCUSSION

Among the four hydrotropes selected for the present investigation aromatic NaNBBS and NaCS exhibited remarkable property of selective extraction

of embelin from *E. ribes* berries. The purity and recovery of embelin in NaPTS and NaXS were very insignificant (data is not shown). The solubility of embelin increased with increasing hydrotrope concentration. Hydrotropy was operational above the MHC of NaNBBS and NaCS (0.1 mol/l). The interaction of embelin with NaNBBS seems to be slightly higher than that of NaCS. Maximum percentage (95%) of extraction and 92% purity was achieved in 3 hr with NaNBBS at 3 mol/l concentration (Fig. 2). Whereas NaCS was less effective with a 92% recovery rate of embelin with 90% purity recorded at 3 hr (Fig. 3). The percentage of extraction is defined as the percentage of embelin initially present in the raw material that was extracted into a hydrotrope solution.

The alkyl benzene sulfonates have a definite and substantial effect on the solubility of embelin, which increased progressively with the hydrotropic concentration and the highest was recorded with NaNBBS at 3 mol/l. The differences in hydrotropy can be attributed to the different sizes of their hydrophobic parts, number of $-CH_2$-groups in the hydrocarbon side chains, and efficiencies of intermolecular packing in their self aggregates (25). Hydrotropy or hydrotropic solubilization is a collective molecular phenomenon involving the co-aggregation of solute with hydrotropic molecules and self-aggregation of hydrotropic molecules in aqueous solutions. The size of hydrophobic region of the hydrotrope aggregates is not well known but dissolved solutes experience a reduced micro polarity and increased micro viscosity in hydrotropic solution (15). The hydrophobic region of the hydrotrope aggregates seems to be accommodating the dissolved solutes. The hydrophobic region (v, $Å^3$) provided by a hydrotrope can be estimated from its effective carbon chain length. Hydrophobic volumes were reported to be 215.7 $Å^3$ for NaNBBS, 188.8 $Å^3$ for NaCS, 161.9 $Å^3$ for NaXS and 135 $Å^3$ for NaPTS. The increased solubilization with increasing hydrophobic volume indicates that hydrotropic

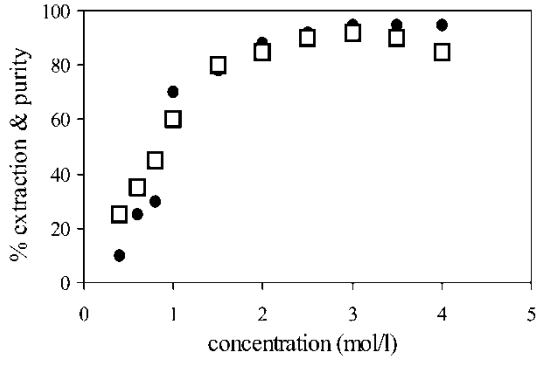

Figure 2. Effect of concentration of NaNBBS on extraction of embelin. ●:- % extraction, □:- % purity.

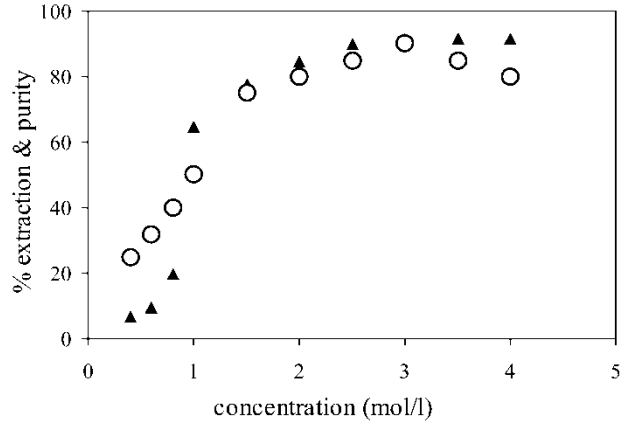

Figure 3. Effect of concentration of NaCS on extraction of embelin. ▲:- % extraction, ○:-% purity.

solubilization could be a consequence of the hydrophobic domains present with in the hydrotrope aggregates (25). The solubilization capacity of a hydrotrope is governed by hydrophobic functionality, i.e. the alkyl group on the aromatic sulfonates. The hydrophobicity of the aromatic sulfonates increases with increasing alkyl group length, they display an increasing tendency for the solubilization of non polar molecules (25). The distinct relationship between the hydrophobic chain length of a hydrotrope and the efficiency of extraction of embelin was in the order of NaNBBS > NaCS > NaXS > NaPTSA.

The hydrotrope used in the experiment have the same polar group, NaNBBS and NaCS were very effective in penetration in the cell wall and extraction of embelin. According to the model proposed by Raman and Gaikar (19) for hydrotropic extraction of natural products the hydrotrope should first adsorb into the cell surface and then penetrate inside the cell wall. The adsorption of hydrotrope on the cell wall helps the reduction of surface forces and improves the cell wall permeability and thus hydrotrope molecules can easily penetrate into the cellulose membrane. The penetration of hydrotrope inside the cell wall induces molecular disorganization and alters the permeability of the membrane by dissolving the cell wall compounds (20). Hydrotrope plays an important role in disorganizing and disordering liquid lamellar structures and can alter the permeability of the membrane and thus embelin is accessible to extract in the hydrotrope solution. The phenomena of hydrotropic extraction can be initiated by adsorption of hydrotropes on the plant cell wall, penetration into the cells, and then solubilization of the active compound (19). For the penetration of hydrotrope into the cellular matrix a lower surface tension is useful in overcoming the surface capillary forces

within the cellular surface. The reported surface tension values of hydrotrope solutions are 50–53 dyn/cm for NaPTS and NaXS, 43 dyn/cm for NaCS, and 40.3 dyn/cm for NaNBBS at their minimum hydrotropic concentration (15). It may be concluded that NaNBBS and NaCS have good penetrative capacity compared to weak hydrotropes such as NaXS and NaPTS.

In a higher concentration of hydrotropes a high osmotic pressure develops across the cell wall and thus at this higher concentration the hydrotrope solution enters the cell wall relatively slow and enables the solubilization of other cellular components. This in turn affects the solubilization of embelin in the hydrotropic solution. Thus in both the cases of NaNBBS and NaCS there was a reduction in the extraction rate of embelin when the concentration increased from 3 mol/l to 4 mol/l. Further studies have conducted at 3 mol/l concentration of hydrotropes.

Effect of Temperature on Extraction

Figures 4 and 5 show a comparison profile of embelin with NaNBBS and NaCS solubility at 40°C and 50°C of temperatures for 3 hr. As the time increased the percentage of yield became constant as the extraction reached a saturation level. In the case of NaNBBS at 40°C the extraction of embelin was 95% with reduced purity of 65%. At 50°C the maximum extraction was recorded at the cost of purity. At 40°C and 50°C the extraction of embelin with NaCS were recorded 95% and 98% respectively with reduced purity.

Inorganic phosphorous in NaBBS and NaCS extracts at 28 ± 2°C was estimated at the range 0.0132 to 0.0143 mol/l. At elevated temperatures of 40°C and 50°C, the presence of phosphorous in the hydrotrope extracts was estimated at 0.0201 to 0.0212 mol/l and 0.0256 to 0.0321 mol/l respectively

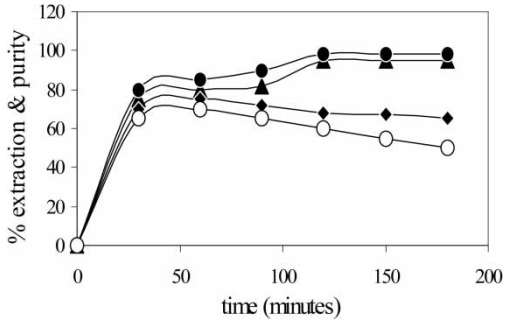

Figure 4. Effect of temperature on the extraction of embelin by NaNBBS. ●:- % extraction at 50°C, ▲:-% extraction at 40°C, ◆:-% purity at 40°C, ○:-% purity at 50°C.

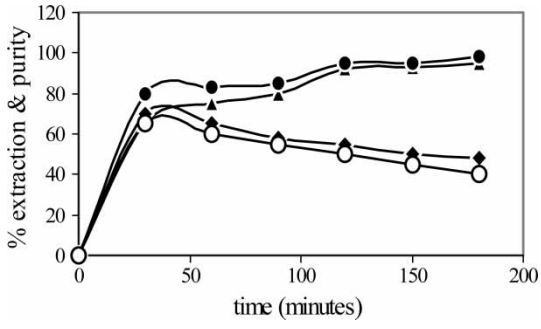

Figure 5. Effect of temperature on the extraction of embelin by NaCS. ●:- % extraction at 50°C, ▲:-% extraction at 40°C, ◆:-% purity at 40°C, ○:-% purity at 50°C.

indicating the lysis of the cell structure at a higher temperature. Thus the cell wall became more permeable to the hydrotrope solution. This facilitates much more diffusion of fatty acids and oleoresins in the solution and subsequently it decreases the selectivity of the hydrotrope solution. At a temperature of 28 ± 2°C probably only the destabilization of the liquid lamellar structure of the cell membrane takes place, which enables more selective extraction of embelin to hydrotrope solutions.

CONCLUSION

Hydrotropic extraction shows a tremendous potential for commercial production of hydrophobic naturally occurring compounds in future, as the process of extraction is economically feasible. A single step process of extraction gives pure embelin with a recovery of 92% as compared to conventional multiple extraction and purification process. The process is simple and rapid and easy to scale up without organic solvents. The use of the aqueous solution avoids possible hazards of conventional extraction and purification involving organic solvents. After the recovery of the compounds the hydrotropic solutions may be reused since there is no chemical reaction between the hydrotrope moiety and the extracted products. The simple recovery step along with no contamination of the product by the hydrotrope and a potential reuse of the hydrotrope solution make the technique economically attractive for the extraction of embelin.

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