

LC-MS & DFT Investigations of *Cryptolepis Sanguinolenta* Root Extracts

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Abstract – The roots of *Cryptolepis sanguinolenta*, a medicinally important ethanobotanical source of the antimalarial cryptolepine, were soxhlet extracted in anaerobic conditions, using hexane then ethanol. Samples of each extract were fractionated using flash chromatography and preparative TLC and compound identity was established using gradient HPLC-positive ion electrospray mass spectrometry. The use of argon depressed the formation of quindoline and hydroxycryptolepine. In addition to known compounds such as cryptolepine, several as yet unidentified compounds remain to be characterised in this root extract.

INTRODUCTION

In West Africa the roots of *Cryptolepis sanguinolenta* are traditionally used to treat malaria and upper respiratory tract infections by making an aqueous decoction of the roots to drink [1]. However, to our knowledge, details of the exact methodology for analysis of *Cryptolepis* extracts, by HPLC-MS, have not been published. In this study, the roots of the plant were extracted and chromatographic methods employed to isolate known compounds e.g. cryptolepine.

MATERIALS AND METHODS

A number of systems were investigated from which chloroform: methanol: ammonia 90:10:1 (v/v/v) was chosen for medium pressure (N₂ gas) flash chromatography on silica gel. Analysis and combination of 160 fractions, using UV excitation at 254 and 366 nm (fluorescent indicator), gave 32 fractions that were analysed on preparative TLC. Spots on the baseline were further separated by switching the solvent composition from chloroform: methanol: ammonia 90:10:1 to 70:30:1 (v/v/v). A C18 X Bridge HPLC column (2.1 × 50 mm, particle size of 3.5 μm) was used in conjunction with a Waters 2695 separation Module and masses were detected in positive ion mode at high resolution (HRPIESMS), using a Waters 486 tunable absorbance detector and a Waters Micromass LCT Classic. Density functional theory (DFT) calculations were performed using published methodology [2]. Purified cryptolepine was incubated with hemin chloride in methanol water at various apparent pH values and analysed with HRPIESMS.

RESULTS AND DISCUSSION

On comparing the four soxhlet extracts, the masses corresponding to cryptolepine and, its isomers, were present in a

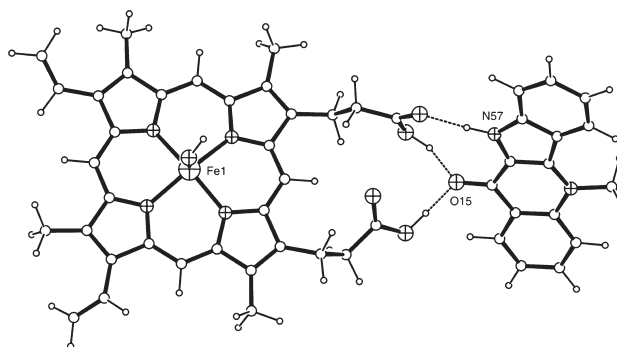


Fig. 1. An optimised structure of hematin-hydroxycryptolepine complex (keto tautomer) in edge binding mode. Hydrogen bonds shown as dotted lines are 1.761, 1.570, 1.570 Å reading from top to bottom. HRPIESMS, e.g. pH 14, C₅₀H₄₄FeN₆O₅⁺; predicted: 864.2717; Found: 864.2710 Da.

higher % abundance in ethanol extracts (fresh and one year old) than in hexane extracts. In contrast, the masses relating to quindoline, hydroxycryptolepine (and its tautomer), cryptoleptine, 11-isopropylcryptolepine and cryptolepicarboline each showed lower % abundances in ethanol extracts. The remaining compounds, cryptomisine and its isomer, cryptoleptine as well as cryptoleptine each showed similar % abundance within the four extracts.

Incubation of purified cryptolepine with hemin chloride at apparent pH 14, pH 7 and pH 2 (methanol/water, HCl, O₂) indicated conversion of cryptolepine (7 h) to hydroxycryptolepine in solution (HRPIESMS). DFT experiments revealed that edge binding (as opposed to above or below face) structural motifs previously considered important for the bisquinoline metaquine [3] (molecular mechanics study) [4], was enhanced by hydroxylation of cryptolepine (Fig. 1), suggesting that cryptolepine acts as an antiplasmodial prodrug, undergoing conversion to hydroxycryptolepine, both in the host liver and within *Plasmodium falciparum* food vacuoles. Poor solubility of these drug receptor complexes at low pH has, until now, excluded investigations at acidic apparent pH values close to those found in parasite food vacuoles.

CONCLUSIONS

A novel flash chromatography/preparative TLC method has been developed to isolate compounds from *Cryptolepis sanguinolenta*. Cryptolepine isolated from freshly ground root interacts with hematin in air and produces hydroxycryptolepine, suggesting prodrug action within parasite vacuoles; a process that can be readily detected using HRPIESMS.

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Antihyperlipedemic Effect of the Seabuckthorn Berry Extract, a Comparative In Vivo Study in Rabbits

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INTRODUCTION

Seabuckthorn (*Hippophae rhamnoides*, *Elaeagnaceae*) is a naturally grown bush which is widely distributed in the mountain regions of Asia and Europe [1]. Several studies have been carried out on different parts of the plant [2], particularly on its berries. The berries have been reported as an effective adjunctive to the cancer treatment [3], to help improving cardiovascular risk factors [4], beneficial for gastrointestinal ulcers and for variety of skin disorders [5–6] and liver cirrhosis.

MATERIALS AND METHOD

Seabuckthorn berries were dried, pulverised and extracted from alcohol after a three day maceration process. Rabbits were made hyperlipidemic with oral administration of 250 mg/kg BW cholesterol filled in hard gelatin capsules for 7 days. The induction of hyperlipidemia was confirmed with the measurement of serum cholesterol, triglyceride (TG), low density lipoproteins (LDL) and high density lipoproteins (HDL) levels using randox and human kit method. The hyperlipidemia was maintained in animals by continued administration of 250 mg/kg BW cholesterol for the entire study period. After 7 days, the rabbits were randomly divided into groups I to V, each having 6 animals.

Total serum cholesterol, TG, LDL, and HDL levels were measured after different treatments on day 0 to day 35 with intervals of 7 days. An emulsion containing 100 mg/ml of extract in distilled water was prepared by dry gum method. The emulsion was administered orally at a dose of 500 mg/kg BW of the animal in group I. Vitamin C (30 mg/kg BW), vitamin E (30 mg/kg BW), atorvastatin (3 mg/kg BW) and sample vehicle (control) as empty hard gelatin capsule shells were administered to group II, group III, group IV and V, respectively. Change in base line values of the above hyperlipidemic markers after all treatments were compared using *post hoc* Tukey's test using SPSS version 13 (Evaluation version). A *p* value <0.05 was considered as significant difference.

RESULTS AND DISCUSSION

There was a marked decrease of $72 \pm 4\%$ in serum cholesterol at day 28 by atorvastatin. The extract, vitamin C and vitamin E, demonstrated a reduction in cholesterol as $69 \pm 4.04\%$, $52 \pm 7.21\%$, and $58 \pm 4.7\%$, respectively. In the case of serum LDL levels, atorvastatin exhibited the highest percentage reduction ($65 \pm 4.2\%$), followed by $56 \pm 5.7\%$ reduction with SBT extract, $50 \pm 6.5\%$ by vitamin E, $48 \pm 11.3\%$ by vitamin C and $33 \pm 4.6\%$ by the control. Similarly, a marked decrease in TG was observed as $70 \pm 3.5\%$ by atorvastatin, followed by $57 \pm 8.1\%$ with the extract, $51 \pm 3.8\%$ by vitamin E, $47 \pm 11.2\%$ by vitamin C, and $29 \pm 7\%$ by control. On the contrary, vitamin C was considered to increase the HDL levels with the highest value of $51 \pm 5.1\%$. Serum HDL levels gradually increased significantly with the treatment of extract ($49 \pm 19.8\%$) as compared to $45 \pm 4.7\%$ with vitamin C, $19 \pm 5.9\%$ with atorvastatin and $5 \pm 1.6\%$ with control respectively from day 7 to day 28, until the end of the total period of study.

CONCLUSION

The findings of the present study indicated that the extract had remarkably decreased the levels of total cholesterol, LDL, and TG as compared to the well known antioxidant vitamins such as vitamin E and vitamin C. The extract may be of value in lowering the cardiovascular risk factors in human related to hyperlipidemia.

KEY WORDS

Sea buckthorn, Low density lipoprotein, High density lipoprotein, Triglyceride.

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