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Tryptophan fluorescence spectroscopy: key tool to study protein denaturation/anti-inflammatory assay

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ABSTRACT

Tissue inflammation is always associated with protein denaturation which in turns is associated with indications like redness, pain, heat, swelling and loss of function at that area of tissues. The present cited Tryptophan fluorescence spectroscopy as a key tool to study protein denaturation/anti-inflammatory assay. BSA was chosen a key protein to study inhibitor effect of herbal derivatives like essential oil and herbal tea. It was observed that all herbal derivatives substantial inhibited protein denaturation under in vitro conditions. It was concluded that Tryptophan fluorescence spectroscopy is a key tool by which researchers can study protein denaturation under lab conditions and can be added as key practical in UG and PG classes.

INTRODUCTION

Increase in the prevalence of multiple drug resistance has shown the way in development of new anti-inflammatory drugs from alternative sources (1). Inflamation is a complex biological response of the vasculer tissues to harmful stimuli (2). When cells in the body are damaged by microbes, physical agents, or chemicals agents, the injury is in the formd of stress. Inflamation of tissue is due to response to stress. The major cause of tissue inflammation is protein denaturation which is associated with indications like redness, pain, heat, swelling and loss of function at that area of tissues (3). The major reason behind loss of protein functions is the disruption of hydrogen, hydrophobic and disulphide bonds in protein structures. Hence it was deduced that compounds which are able to prevent these changes and inhibit heat induced protein denaturation have potential to be used as therapeutic anti-inflammatory drugs. Eukaryotic and prokaryotic cells contain a number of compounds that are fluorescent when excited with UV light. Most important endogenous flurophores are molecules widely distributed in cells and tissues, like proteins containing aromatic amino acids. Many enzymatic cofactors, such as FMN, FAD, NAD and porphyrins, which are also intrinsically fluorescent, add to the protein fluorescence play important roles in the cellular energy metabolism (4). The

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experiment demonstrated here have been developed as a part of overall revision of advanced courses in biochemistry and biotechnology which highlight the importance of physical and analytical chemistry to study the structures and properties of biological macromolecules. The experiments reported here involve the use of tryptophan fluorescence spectroscopy to determine the protein denaturation or anti-inflammatory studies.

METHODS

Protein denaturant assay was also studied using fluorescent assay. The reaction mixture contained 0.4ml of 1% BSA, 4.78 mL of phosphate buffered saline (PBS, pH 6.4) and 100 µl of *Eucalyptus globulus* essential oil, Lemon grass tea and nano-emulsions. The reaction mixture was incubated in water bath at 37°C for 15 min. After that reaction mixture was heated at 70°C for 5 min. After cooling, 1 mL of mixture was subjected to fluorescent spectroscopy analysis on Perkin Elmer Spectrophotometer (FL6500). The excitation wavelength was 280 nm and fluorescence emission spectrum was recorded over wavelength range from 300-400 nm. All experiments were done at room temperature (~30^{o}C).

RESULTS AND DISCUSSION

In order to study protein denaturation process, bovine serum albumin (**BSA**) was chosen. BSA, like most globular proteins, contains aromatic amino acids. The three amino acid residues that are primarily responsible for the inherent fluorescence of proteins are tryptophan, tyrosine and phenylalanine (1). These amino acids are known to be fluorescent, but tryptophan (Trp-134, Trp-213, Trp-214) fluorescence dominates usually the fluorescence of the macromolecules. Furthermore when, when BSA is denatured, the exposure of tryptophan residues in the hydrophobic core of the molecules results in the fluorescence increase. Hence the fluorescence intensity may be then be used as a measure of the extent of unfolding of the protein.

In the present study protein denaturation inhibitory study of herbal derivatives like eucalyptus essential oil, lemon grass tea and some oil based nano-emulsions was investigated by fluorescent technique. Therefore, in order to study the inhibitory effect of all herbal derivaties on protein denaturation process, bovine serum albumin (BSA), a water soluble protein, was chosen. Typical fluorescence spectra for denatured BSA and BSA plus herbal derivatives are shown in Fig. 1A/B. All derivaties exhibited substantial decrease in inhibition of protein denaturation, as fluorescence intensity decreased substantially after the addition of derivaties to BSA. The fluorescence intensity using commercial synthetic drug asprin is also shown in Fig. 1A.To conclued this study has highligted a key tool tryptophan fluorescence spectroscopy to study protein denaturation assay

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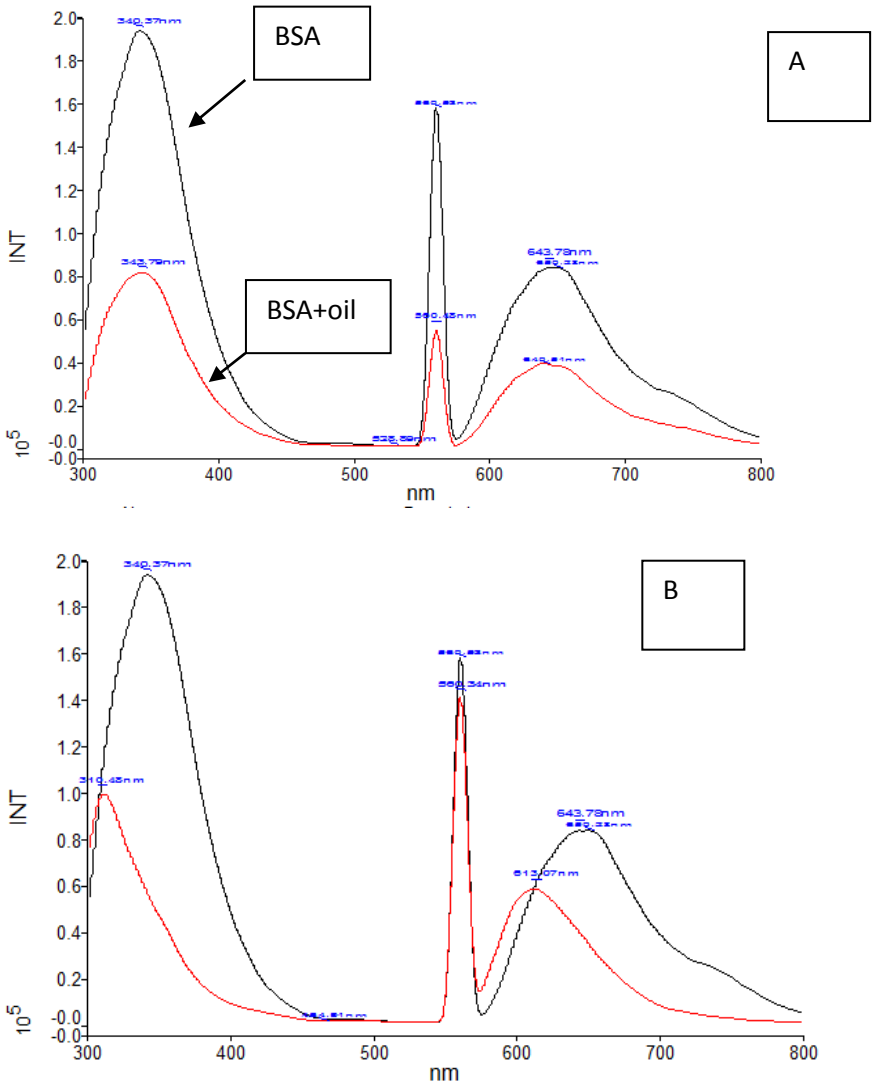

Figure. 1A: Anti-inflammatory (protein denaturation activity/BSA denaturation assay) activity of Eucalyptus oil (A) and Asprin (B) using fluorescent technique

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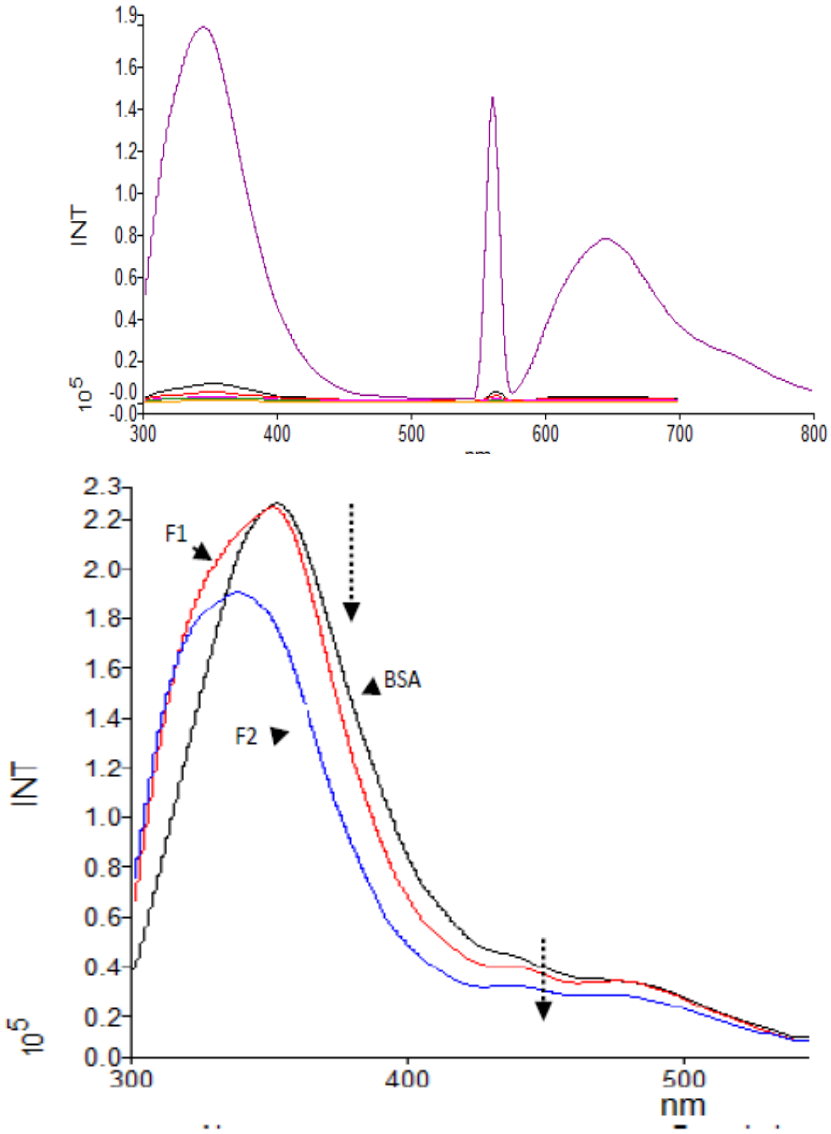

Fig 1B: Protein denaturation (tryptophan fluorescent spectroscopy) analysis of nanoemulsions (A), Lemon grasss tea (B).

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CONFLICT OF INTEREST

No

REFERENCES

1. Kumar V, Singh S et al. Determination of phytochemical, antioxidant, antimicrobial, and protein binding qualities of hydroethanolic extract of Celastrus paniculatus. Journal of Applied Biology & Biotechnology 6 (06); 2018: 11-17
2. Talamond, P. Verdeil JL, Conejero G. 2015. Secondary metabolite localization by auto-fluorescence in living plant cells. Molecules, 20, 5024-5037.
3. Tlili N, Khaldi A, Triki S, Munné-Bosch S. Phenolic compounds and vitamin antioxidants of caper (*Capparis spinosa*). 2010: Plant Foods Hum. Nutr. 65: 260–265.