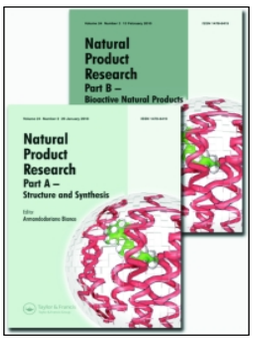

Natural Product Research

Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: http://www.tandfonline.com/loi/gnpl20

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To cite this article: Wajiha Ahmad, Nosheen Fatima Rana, Sundus Riaz, Nasir Mehmood Ahmad, Maryam Hameed, Ayesha Naeem & Rabbiya Tahir (2018) Chemical sensing of Benzo[a]pyrene using Corchorus depressus fluorescent flavonoids, Natural Product Research, 32:8, 968-971, DOI: 10.1080/14786419.2017.1367778

To link to this article: https://doi.org/10.1080/14786419.2017.1367778

NATURAL PRODUCT RESEARCH, 2018
VOL. 32, NO. 8, 968–971
https://doi.org/10.1080/14786419.2017.1367778

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SHORT COMMUNICATION

Chemical sensing of Benzo[a]pyrene using *Corchorus depressus* fluorescent flavonoids

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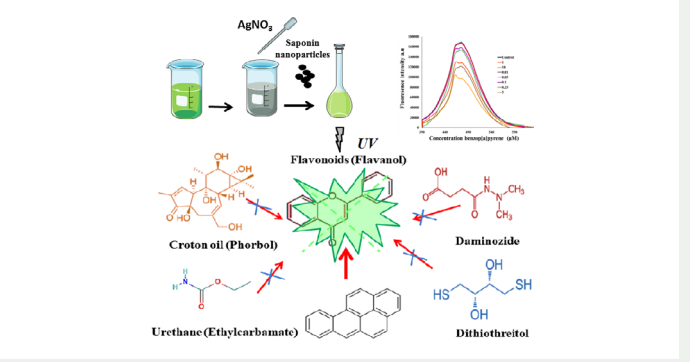
ABSTRACT

Plant phytochemicals, such as flavonoids are in use for the development of optical biosensor. Benzo[a]pyrene (B[a]P), is a pervasive environmental and dietary carcinogen. A fluorescent assay is developed using plant isolated flavonoid for the detection of B[a]P. High content saponins are excluded from the flavonoid-containing methanolic extract of *Corchorus depressus* by implying reduction of silver ions by saponins resulting in formation of silver nanoparticles. Isolated plant flavonoids are used to develop a spectrofluorometric assay for the detection of B[a]P. Decrease in the flavonoid fluorescence intensity by B[a]P is found to be based on both static and dynamic quenching. Specificity of the assay for B[a]P was tested for other carcinogens belonging to different classes of compounds. Flavonoids-mediated sensing can be implied for the development of new generation of nanoparticle-based biosensors that can be more sensitive and less susceptible to external factors, such as temperature and humidity.



ARTICLE HISTORY
Received 28 April 2017
Accepted 5 August 2017

KEYWORDS
Benzo[a]pyrene; *Corchorus depressus*; fluorescence quenching; flavonoids

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) constitute a large group of organic adulterants. They are comprised of carbon and hydrogen forming 2–7 condensed benzene rings (Yang

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Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2017.1367778.

et al. 2010). PAHs are formed by incomplete burning of organic compounds, inadequate storage and removal of fuels, oils and wood treatment practices (Shemer and Linden 2007). They are highly stable hydrophobic molecules that easily interact with cell membranes and accumulate in lipid tissues. International agency for research on cancer (IARC) has classified these molecules as potent carcinogens due to their proved mutagenic and carcinogenic properties (Del Carlo et al. 2012). Acquaintance to B[a]P can also have adverse effects on nervous, reproductive, immunological and endocrine systems. Conventional methods used for B[a]P analysis include enzyme-linked immunosorbent assay (ELISA), gas chromatography–mass spectrometry (GC-MS), high-pressure liquid chromatography (HPLC) are precise and sensitive but they need tiresome radio labelling methods and are time consuming (Murphy and Hecht 1985; Haugen et al. 1986; Gündel and Angerer 2000). Recently, various other assays are developed for the quantification of B[a]P. However, they require multi-step processing and expensive chemicals. These include molecularly imprinted solid-phase extraction (MISPE) coupled with ELISA (Pschenitza et al. 2014), electro-switchable bio surfaces developed by associating anti-B[a]P antibodies (Lux et al. 2015), ultrasound-assisted liquid–liquid extraction (Taghvaee et al. 2016) and fluorescence enhancement and FRET-based immunoassay (Li et al. 2016).

Corchorus depressus belong to Tiliaceae family and is used as folk medicine. Roots and branches of this plant are rich in saponins; however, other glycosides and flavonoids are also present. In present study, fluorescent flavonoids are isolated from *C. depressus* methanolic extract by the formation of saponin silver nanoparticles. Isolated flavonoids emitted yellow green fluorescence in ultraviolet light. A fluorescence quenching assay is developed for the quenching of these flavonoids by B[a]P.

2. Results and discussion

Phytochemicals present in plant extract act as reducing agents for metal ions and are of great importance in green synthesis of metallic nanoparticles of therapeutic importance (Ahmad et al. 2010). One of the applications of the reducing property of phytochemicals can be the isolation and separation of plant secondary metabolites. Methanolic extract of *C. depressus* branches is rich in saponins and flavonoids are present in very low percentage. Upon addition of silver nitrate, saponin silver nanoparticles are formed, see supplementary material. Saponin silver nanoparticles were harvested and a fluorescent emitting supernatant-containing flavonoids was obtained. Flavonoid determination was done through Shinoda's test and alkaline reagent test (Figure S1). Quantitative determination of flavonoids was done by calorimetric analysis presenting bathochromic shift, using Quercitin as standard. Supernatant was found to have 89 ± 16 µL of flavonoids. FTIR spectra of flavonoids are shown in Figure S2.

B[a]P itself is a fluorescent-emitting carcinogen and its emission spectra lies in the same region as that of plant flavonoids. The absorption spectra of the plant flavonoids and B[a]P (Figure S3) were analysed in order to obtain the excitation wavelength, where plant flavonoids are excited and B[a]P is not. The excitation wavelength of 330 nm is selected for the assay and emission at 465 nm was selected.

Assay response to various concentrations of B[a]P are shown in Figure S4, which shows that addition of varying concentration of B[a]P to the flavonoid decreased the fluorescence intensity. Type of quenching is determined by fluorescence quenching spectra of substance

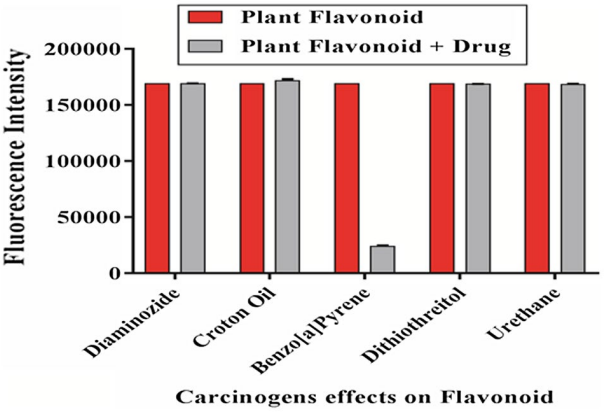

Figure 1. Complexion behaviour of plant flavonoid with different drugs.

and the absorption spectra of fluorescence substance in the presence and absence of quencher. Stern–Volmer plot (Figure S5) shows the fluorescence quenching spectra and the upward curvature indicates combined quenching (static and dynamic) existence (Gong et al. 2007).

The selectivity and specificity of sensing agents in the presence of different compounds in detection media leads to development of a specific chemo sensor. The selectivity of plant-mediated flavonoids for quenching B[a]P was tested in the presence of four other carcinogens belonging to different classes (Figure 1). In this context, croton oil (phorbol), urethane (ethylcarbamate), daminozide and dithiothreitol were applied for quenching. The fluorescence intensity of flavonoids was not affected by any of the other carcinogens.

3. Conclusion

A robust and cost-effective assay is developed for the detection and quantification of benzo[a]pyrene. The assay does not imply any extraction and/or antibody or aptamer requirement. *C. depressus* flavonoids can be further characterised and is used for the fabrication of simple and inline biosensors, such as paper-based fluorescent biosensors. Further studies are required to assess the interaction of these flavonoids with other polycyclic aromatic hydrocarbons.

Disclosure statement

No potential conflict of interest was reported by the authors.

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