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Original article

Analysis of antioxidative and antiviral biomarkers β-amyrin, β-sitosterol, lupeol, ursolic acid in *Guiera senegalensis* leaves extract by validated HPTLC methods

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ABSTRACT

Guiera senegalensis J.F. Gmel is a broad-spectrum African folk- medicinal plant, having activities against fowlpox and herpes viruses. Very recently, we have shown the anti-hepatitis B vius (HBV) potential of *G. senegalensis* leaves extract (GSLE). Here, we report the antioxidative and hepatoprotective efficacy of GSLE, including HPTLC quantification of four biomarkers of known antioxidative and antiviral activities. In cultured liver cells (HuH7) GSLE attenuated DCFH-induced oxidative stress and cytotoxicity. This was supported by *in vitro* DPPH radical-scavenging and β-carotene-linoleic acid bleaching assays that showed strong antioxidant activity of GSLE. Further, two simple and sensitive HPTLC methods (I and II) were developed and validated to quantify β-amyrin, β- sitosterol, lupeol, ursolic acid in GSLE. While HPTLC-I (hexane: ethylacetate; 75:25; v/v) enabled quantification of β-amyrin (Rf = 0.39; 20.64 μg/mg) and β-sitosterol (Rf = 0.25; 18.56 μg/mg), HPTLC-II (chloroform: methanol; 97:3; v/v) allowed estimation of lupeol (Rf = 0.47; 6.72 μg/mg) and ursolic acid (Rf = 0.23; 5.81 μg/mg) in GSLE. Taken together, the identified biomarkers strongly supported the antioxidant and anti-HBV potential of GSLE, suggesting its activity via abating the oxidative stress. To our knowledge, this is the first report on HPTLC analysis of these biomarkers in *G. senegalensis* that could be adopted for standardization and quality-control of herbal-formulations.

1. Introduction

Oxidative stress-induced cellular injury is caused by the imbalance between the oxidant and antioxidant molecules or by over-abundance of reactive oxygen species (ROS), produced by endogenous or exogenous sources (Opara et al., 2006). The accumulating excess of ROS can damage lipids, proteins or nucleic acids, and inhibit the normal growth and function of the cells (Ames et al., 1993). Several *in vitro* and *in vitro* studies have suggested the association of oxidative stress and damages with different forms of liver diseases (Ha et al., 2010). The hepatitis B virus (HBV) infection results in acute and chronic liver diseases, such as hepatitis B, cirrhosis and hepatocellular carcinoma (HCC). Evidences have shown that HBV can induce oxidative stress *in vitro* and *in vivo* including chronic hepatitis B patients (Severi et al., 2006; Niu et al., 2009; Bolukbas et al., 2005). Notably, the oncogenic 'X' gene of HBV (HBx) is trans-activated by ROS, and plays a crucial role in the development of HCC. Moreover, the polyunsaturated fatty acids residues of phospholipids of cell membranes and intracellular organelles are highly reactive to ROS that lead to lipid peroxidation (LPO), and produce cytotoxic malondialdehyde and hydroxynonenal (Djordjevic, 2004). It is reported that the total peroxide level, LPO and oxidative DNA damage are significantly higher in hepatitis B patients (Shaban et al., 2014).

Currently, the use of medicinal plants is massively increasing due to fewer or insignificant side effects as well as its low-cost.

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The African medicinal plant, *Guiera senegalensis* J.F. Gmel, commonly known as 'Cure all' is a popular folk medicine in the treatment of several types of metabolic and infectious diseases (Suleiman, 2015; Bosisio et al., 1997; Somboro et al., 2011). The dried leaves preparations of *G. senegalensis* is used to treat cough, sexual, gastrointestinal, respiratory and skin diseases, including their use as acaricidal, antimalarial, and antimicrobial, antioxidant, anti-inflammatory and gastroprotective agents (Osman et al., 2014; Bouchet et al., 1998; Sombié et al., 2011; Akuodor et al., 2013). Importantly, the galls of the plant are reported to have *in vitro* antiviral efficacies against fowlpox virus (FPV) (Lamien et al., 2005) and herpes simplex virus (HSV) (Silva et al., 1997). Very recently, we have shown the *in vitro* anti-HBV efficacy of *G. senegalensis* leaves (Arbab et al., 2017).

It is known that the pharmacological activity of a herbal-formulation is attributed to bioactive constituents, and their amount can differ considerably depending on the plant's part used, its geographical origin and the season of harvest. Therefore, development of sensitive methods for quantitative analysis of active biomarker(s) in a claimed plant extract or marketed formulation is fundamental for ensuring its therapeutic quality. Therefore, owing to its low-cost, high-throughput and minimum sample clean-up properties, the high-performance thin-layer chromatography (HPTLC) has become a convenient analytical method. The phytochemical analysis of *G. senegalensis* has identified various bioactive flavonoids, alkaloids, tannins and a naphthyl butenone (Ficarra et al., 1997; Bouchet et al., 1996). However, natural triterpenes (the structurally diverse group of pentacyclic triterpenoids) and phytosterols of known antioxidant and antiviral activities are hitherto, not explored in *G. senegalensis*. The present study was therefore, designed to evaluate the antioxidative and hepatoprotective property of anti-HBV active *G. senegalensis* leaves ethanol-extract (GSLE) and, to quantify biomarkers (β-amyrin, β-sitosterol, lupeol, ursolic acid; Fig. 1) by validated HPTLC methods.

2. Experimental

2.1. Plant material and extract preparation

Fresh and clean leaves of *G. senegalensis* (Family: Combretaceae) were collected from Kordofan region, and authenticated (voucher specimen no. 798) at the Forestry Research Center (FRC), Khartoum, Sudan. A further verification was done by a taxonomist at the herbarium of College of Pharmacy, King Saud University, Saudi Arabia. The leaves were washed and dried at room temperature for a week and powdered (50 g) using mortar-pestle. The extraction was done with 500 mL of 70% ethanol (Merck) for 24 h with intermittent shaking, and repeated twice with fresh solvent. The extracts were pooled, passed through Whatmann filter paper, and dried under reduced pressure using rotary evaporator (R-210, BUCHI).

2.2. Antioxidant activity assays of GSLE

2.2.1. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The antioxidant activity was tested by DPPH radical scavenging ability of GSLE in a 96-well microplate as described elsewhere (Bouchet et al., 1998; Wong et al., 2014). Briefly, triplicates of 100 µL of GSLE (0.0, 31.25, 62.5, 125, 250 and 500 µg/mL) was mixed with 50 µL of 0.2 mM DPPH (Sigma, USA) in a 96-well flat-bottom microplate (Becton-Dickinson Labware, USA). Rutin, an antioxidant natural flavonoid was used as positive control. Following a 30 min incubation at 25 °C in dark, the absorbance (λ= 517 nm) was recorded using microplate spectrophotometer (BioRad, USA). The data was analyzed for radical scavenging activity of SGEE, using the following equation:

$$\%\textit{Radical scavenging activity} = (1 - As/Ac) \times 100$$

[As : absorbance of sample; As : absorbance of control].

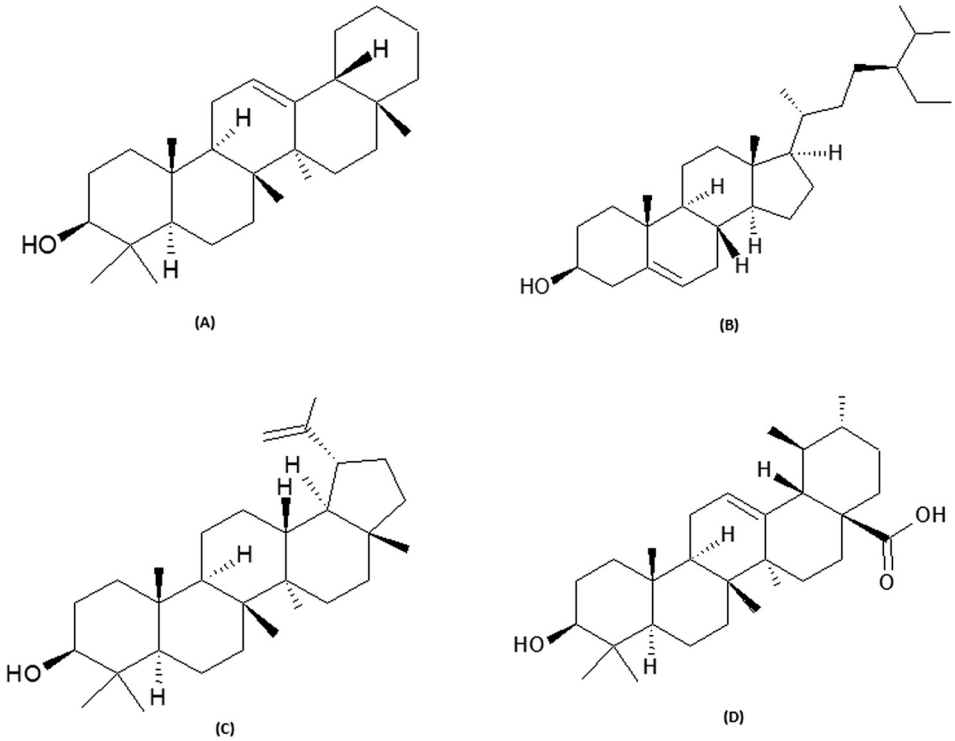

Fig. 1. Chemical structures of antioxidant biomarkers analyzed in the present study. (A) β-amyrin, (B) β-sitosterol, (C) lupeol, and (D) ursolic acid.

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