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PHYTOCHEMICAL AND PHARMACOLOGICAL ASPECTS OF ANTHRAQUINONES OF Rheum australe D. *Don*

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ABSTRACT

Anthraquinones are phenolic compounds known best for their laxative activity. Rheum australe D. Don (Indian rhubarb, Polygonaceae) is a prominent medicinal plant with anthraquinones as the bioactive compounds, among a few others. The online literature search was carried out to collect data on the phytochemistry and pharmacological activity of anthraquinones, particularly those isolated from R. australe. Anthraquinones are commonly found in the genera of Rheum, Senna, Aloe, Frangula, and Rubia, which can be quantitatively and qualitatively determined using both conventional and advanced analytical methods. The anthraquinones of R. australe were found in free and glycosidic forms, which were best extracted by the microwave-assisted extraction method. Various chromatographic techniques were commonly conducted to isolate the pure compounds. In addition to its laxative activity, anthraquinones of R. australe also showed potential antibacterial, cytotoxic, and antioxidant properties.

Keywords: Anthraquinones; Pharmacological activities; Phytochemistry; Rheum australe D. Don

1. INTRODUCTION

Rheum australe D. Don (synonym of *Rheum emodi* Wall. ex Meisn., Indian rhubarb, Polygonaceae) is a rhubarb species commonly grown in the higher altitude of India and China. It is cultivated for culinary, ornamental, and medicinal purposes. The plant is extensively used in both Chinese and Ayurvedic folk medicinal systems. The crude drugs prepared from roots and rhizomes are traditionally used for astringent, purgative, tonic, and ulcer healing. The antifungal, antioxidant, hepatoprotective, nephroprotective, and immunomodulatory activities of *R. australe* have been reported (Zargar et al, 2011).

As R. australe is considered as a medicinal- and economically valuable plant, its phytochemistry and pharmacology have been reviewed multiple times (Pandith et al, 2018; Rokaya et al, 2012). In this article, the phytochemical and pharmacological aspects of anthraquinones derived from R. australe are discussed, including the structures, extraction, determination, isolation, and the confirmed pharmacological activities. Unlike the previous reviews that exclusively discussed R. australe, this article also describes the basic phytochemical aspects of anthraquinones. It covers the general structures, botanical distribution, extraction, determination, and isolation of the compounds.

2. METHODS

The data presented in this review were retrieved from online databases PubMed, Sciencedirect, and Springer. The search terms used were as follow: "anthraquinones", *"Rheum australe", "Rheum emodi"*, "phytochemistry", and "pharmacological activity". The operators "AND" and "OR" were applied. The structures of the selected anthraquinones presented in Figure 1 and the name of the plant were taken from the PubChem and The Plant List databases, respectively.

3. RESULTS AND DISCUSSION

3.1. Phytochemistry of Anthraquinones

Anthraquinones are compounds with a central 1,4-diketo-cyclohexa-2,5-diene (quinone) structure connected to peripheral phenyl rings. In most anthraquinones, C-1 and C-8 typically bind hydroxyl groups. Methyl, hydroxymethyl, or carboxyl group may be substituted to C3, while the phenolic or methyl group may be bonded to C-6. Anthraquinones are usually in the form of glycosides in plants and rarely found in free form. They commonly occur as O-glycosides and C-glycosides, but sometimes the unusual C, O-glycosides is also found. Diglycosides occurred when the binding between aglycone and two glycone units present. For example, glucofrangulin showed the presence of glucose and rhamnose, respectively bound at C-8 and C-6 (Bartnik & Facey, 2016; Bone & Mills, 2013).

Distribution of anthraquinones in angiosperm is limited in some families, i.e., Fabaceae, Liliaceae, Polygonaceae, Rhamnaceae, Rubiaceae, and Scrophulariaceae. The genera that are known for rich with these compounds are including Rheum, Cassia, Frangula, Aloe, and Rhamnus. Anthraquinones are also found in small quantities in Polgyonum, Brassica, and Phaseolus. Aloe (*Aloe ferox* Mill. or A. vera (L.) Burm.f., Xanthorrhoeaceae), rhubarb *(Rheum palmatum L., R. australe* D. Don, or *R. officinale* Baill., Polygonaceae), cascara *(Frangula alnus* Mill. or *F. purshiana* Cooper, Rhamnaceae), senna (*Senna alexandrina* Mill., Fabaceae) are the example of popular plants with anthraquinones as the active constituents. Also, they are found in fungi and lichens (Bartnik & Facey, 2016).

The classical Borntrager color reaction can be used to determine the free anthraquinones in crude drugs. In this method, the ethanolic solution of KOH will react with the compounds and produce vivid colors (red or purple), depending on the group analyzed. Other color reagents that can be utilized for this purpose include magnesium acetate in methanolic solution, boro-acetic acid reagent, and sodium tetraborate. These methods can be used quantitatively and qualitatively. However, those reagents react with all free anthraquinones, so they are commonly used to determine the total anthraquinone in crude drugs. The determination of anthraquinones can also utilize Thin-layer chromatography (TLC) and High-Performance TLC (HPTLC). The most frequent stationary phase is silica, while the acidic mobile phase is used with the addition of a little amount of formic acid. Identification of anthraquinones after separation commonly uses NH3 vapors or Borntrager reagent, both induce color changes. High-Performance Liquid Chromatography (HPLC) has been the mainstay for the determination of anthraquinones. All publications available for this matter utilize a reversed-phase liquid chromatography system with a C18 column. The various detection system has been used to analyze anthraquinones with HPLC, including ultraviolet-visible (UV, usually is set at wavelengths of 254, 280, 430, 480, or 500 nm), fluorimetric, electrochemical, and chemiluminescence detector. The use of the hyphenated technique, in which HPLC is coupled to a mass spectrometer (LC-MS), is rapidly adopted. This analysis usually used electrospray ionization (ESI) in the negative mode. Aside from the popular TLC and HPLC, several techniques for the determination of anthraquinone have also been developed. Those methods include capillary electrophoresis (CE), gas chromatography (GC), supercritical fluid chromatography (SFC), infrared spectroscopy (IR), and nuclear magnetic resonance (NMR). Altogether, those methods are powerful in analyzing anthraquinone compounds quantitatively and qualitatively (Duval et al, 2016).

Conventional extraction methods, including maceration and Soxhlet, have been long used to extract anthraquinones from crude drugs. However, those methods use large organic solvent volumes and are time-consuming. The modern methods include solid liquid-, ultrasonic assisted-, microwave assisted-, pressurized liquid-, and supercritical fluid extraction, were developed to overcome the drawback of the traditional ones. Among all those new methods, the use of pressurized liquid- and ultrasonic-assisted seem to give the most promising result. Isolation of anthraquinones commonly used following chromatographic methods includes

counter-current chromatography (CCC), preparative TLC, and also preparative and semi-preparative HPLC (Duval et al., 2016).

Traditional uses as laxative of plants containing anthraquinones, such as aloe, rhubarb, cascara, and senna, have been recognized since ancient times. Their laxative effects are considered as the actions of 1,8-hydroxyanthracene derivates. Besides, anthraquinones have demonstrated hepatoprotective, anti-psoriasis, cytotoxic, anti-inflammatory, immunosuppressive, antimicrobial, diuretic, vasorelaxant, antioxidant, and phytoestrogen activities (Bone & Mills, 2013; Duval et al., 2016).

3.2. Anthraquinones Derived from *R. australe*

Both unbound and glycosidic forms of anthraquinones are the main phytoconstituents of rhizomes of *R. australe* (Figure 1). The identified compounds in this plants are including rhein, chrysophanol, aloe-emodin, emodin, physcion, chrysophanein, emodin glycosides, 6-methyl rhein, 6-methyl aloe-emodin, 10-hydroxycascaroside C, 10-hydroxycascaroside D, 10R-chrysaloin 1-O-β-D-glucopyranoside, cascaroside C, cascaroside D, cassialoin, and revandchinones (Zargar et al., 2011).

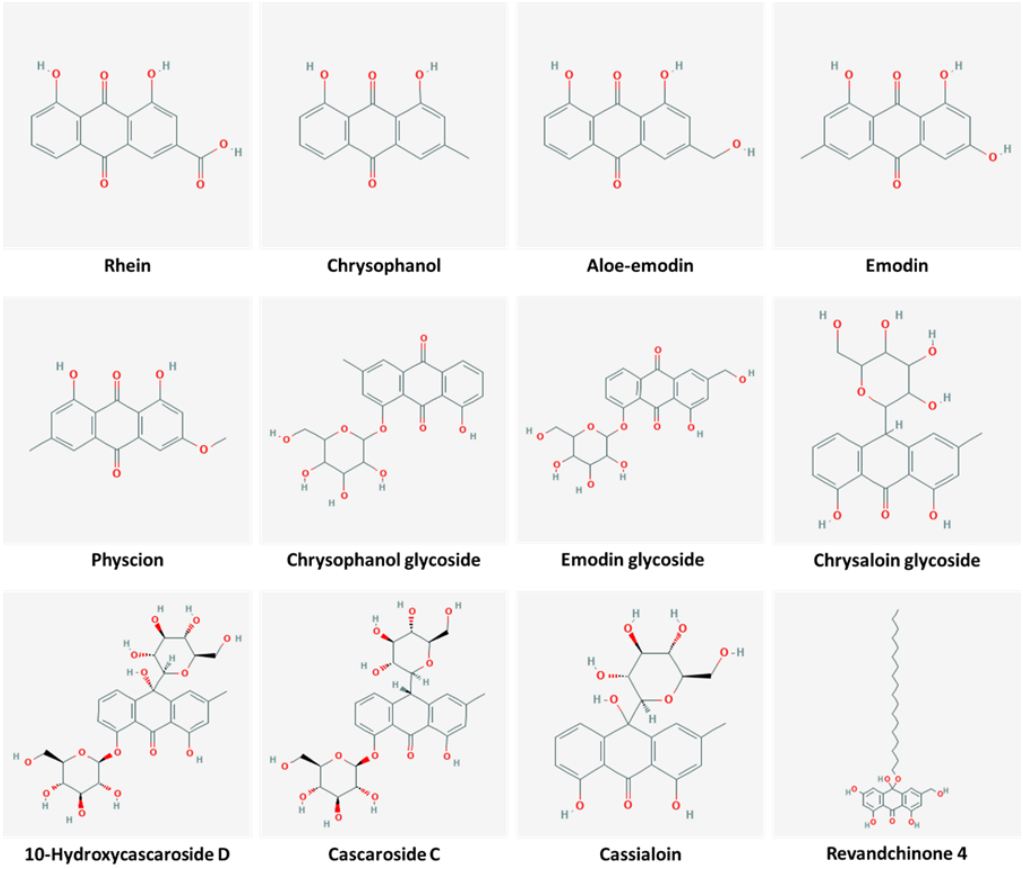

Figure 1. Selected anthraquinones from *R. Australe* (Zargar et al., 2011)

3.3. Phytochemistry of Anthraquinones from *R. australe*

Anthraquinones in *R. australe* can be determined with both traditional and modern techniques as those from another source do. Recently, a study reported the utilization of HPLC-Q-HR/MS for the simultaneous determination of 10 anthraquinones in *R. australe*. The samples are separated over a C18 column at a temperature of 30 ℃. Two mobile phase systems, three mmol/L ammonium acetate in water and methanol, were set in gradient mode for 18 minutes. The hyphenated MS detector utilized electrospray ionization (ESI) technique with the negative ionization full-scan mode. This validated method was proven to be beneficial for the

simultaneous quantification of anthraquinones. Hence, it can be used to standardize *R. australe* crude drugs for medicinal purposes (Zargar et al., 2011).

The 1,8-dihydroxyanthraquinones are commonly used for the marker for the standardization process of *R. australe* crude drugs. A comparison between classical and non-conventional methods showed that aloe-emodin, rhein, emodin, chrysophanol, and physcion were best extracted with ethanol in a plant material - solvent ratio of 1:20. Among all the extraction methods tested, the highest recovery of 1,8-dihydroxyanthraquinones was obtained from heat reflux for 45 min (Aditya U. Arvindekar, Pereira, & Laddha, 2015). In another study, microwave-assisted extraction was proven to be the most suitable method for the extraction of the 1,8-dihydroxyanthraquinones as well as the total anthraquinones in *R. australe* (A. U. Arvindekar, Pereira, & Laddha, 2016).

Although nowadays, isolation of anthraquinones from *R. australe* can be conveniently conducted using CCC, preparative TLC, and preparative HPLC, the classical column chromatography is still being used widely to date. For example, this method was used for isolating dihydroxyanthraquinone derivatives from the rhizome of *R. australe*. The powdered were extracted with methanol in a percolator at room temperature. After filtration, a dark brown mass was obtained from the evaporation of the solvent at a temperature of 60oC. It is then loaded onto an open air-silica gel column and consecutively separated with n-hexane, chloroform, and methanol in a polarity-gradient manner. The eluates were monitored with TLC, and 11 fractions were collected. Fraction 6 (obtained from elution with n-hexane-chloroform (1:3)) was separated further over silica gel column and a mobile phase of the increased polarity of a mixture of n-hexane-chloroform and successfully produced 6-methyl-rhein and 6-methyl-aloe-emodin based on their spectral data (H-NMR, C-NMR, mass and IR spectra) and physical properties (Singh, Pandey, Singh, & Agarwal, 2005).

3.4. Pharmacological Activity of Anthraquinones from *R. australe*

R. australe, along with *R. palmatum* (Chinese rhubarb), has been long used for its laxative activity. Its mechanism of action as a laxative has been well understood. The free anthraquinones act directly on the intestinal wall, while the β-linked glycosides are non-absorbable in the intestine and are metabolized to rhein anthrone by the normal flora in the descending colon. In their respective site of actions, each compound modifies the motility of the large intestine that results in the accelerated colonic transit and the reduced fluid absorption along the process. Later, the compounds change the rate of absorption and secretion of water, retention of potassium, and secretion of active chlorine. Altogether, these processes produced enhanced fluid secretion (Bone & Mills, 2013).

Anthraquinones from *R. australe*, both as active fractions or crude extracts, were mentioned to demonstrate antiviral, antibacterial and antifungal actions, antioxidant and anticancer potentials, nephroprotective, hepatoprotective, antidiabetic, and immuno-enhancing activities, as well as prevention and treatment of Parkinson's disease (Zargar et al., 2011). The newer publications reported the new evidence of the pharmacological activities of *R. australe* anthraquinones. Fractions of *R. australe* grown in Nepal majorly contained chrysophanol and emodin, showed antibacterial, cytotoxic, and antioxidant capacity (Gupta, Bajracharya, & Jha, 2014). The antioxidant potentials of anthraquinones in this plant are developed further into the agricultural application, as they showed potent anti-nematodic activity against *Meloidogyne incognita* (Tripathi et al., 2014). Another study reported that the crude extract, also emodin, chrysophanol, and their respective glycoside forms, decreased the cell viability of various cancer cell lines. The most prominent cytotoxicity was observed on MIAPaCa-2 cell line, while the highly potent cytotoxicity was notably exhibited by crude extract and the free anthraquinones (Pandith et al., 2014).

An in vivo study suggested that chrysophanol, emodin, chrysophanol 8-O-β-D-glucopyranoside, and emodin 8-O-β-D-glucopyranoside of *R. australe* were significantly ameliorating the in-vivo lipid levels and exhibited reactive species scavenging activity in the thiobarbituric acid model. Among those isolated compounds, emodin demonstrated the best lipid-lowering activity (Mishra et al., 2014). Rhein, aloe-emodin, emodin, chrysophanol, and physcion showed a promising anti-hyperglycemic property in the diabetic rats, with aloe-emodin as the most-pronounced compound in decreasing lowering the blood glucose level. In addition, emodin significantly inhibited α-glucosidase, in which the IC50 was lower than that of acarbose (A. Arvindekar et al., 2015).

R. australe alleviated the primary dysmenorrhoea in humans. In a single-blind randomized controlled clinical study involving 45 participants, treatment with capsules containing *R. australe* powders two times a day for five days was sufficient for reducing the symptoms without any apparent side effects. This treatment resulted in decreasing the duration of pain as well as improving the quality of life of the participants (Rehman et al., 2015). *R. australe* was considered safe. In the acute and sub-chronically toxicity studies, the treatment with freeze-dried water extract of the rhizome of this plant at the maximum dose of 4000 mg/kg/day showed no-observed-adverse-effect in both male and female rats (Ye, Feng, & Wang, 2014).

4. CONCLUSION

R. australe is proven to be a valuable plant with a diverse pharmacological spectrum. Its broad activity is mainly underlined by its anthraquinones content. The chemistry, biology, and also pharmacology of anthraquinones of *R. australe* have been studied, and it may support its use beyond the traditional ones.

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