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Original Article

Extraction optimization of gallic acid, (+)-catechin, procyanidin-B2, (−)-epicatechin, (−)-epigallocatechin gallate, and (−)-epicatechin gallate: their simultaneous identification and quantification in *Saraca asoca*☆

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ABSTRACT

The objective of the present investigation was to optimize extraction conditions for maximum recovery of bioactive phenolics from different parts of *Saraca asoca*. Extraction recovery was optimized using a mixture of methanol and water in different proportions. For identification and quantification of six analytes, a rapid reversed phase ultra-performance liquid chromatography (UPLC) photo diode array detection method was developed. UPLC separation was achieved in a gradient elution mode on a C_{18} column with acetonitrile and aqueous phosphoric acid (0.1%, pH = 2.5). Extraction solvent for maximum recovery of analytes varied depending on the nature of matrices. The developed UPLC method was validated in accordance with International Council for Harmonisation (ICH) guidelines. Wide linearity range, sensitivity, accuracy, short retention time, and simple mobile phase composition implied that the method could be suitable for routine analysis of all six analytes with high precision and accuracy. The uniqueness of this study is the determination of the distribution of these compounds in the various parts of *S. asoca*.

1. Introduction

Saraca asoca (Roxb.) De Wilde (Syn. *S. indica* Linn.), which belongs to the family Caesalpiniaceae, is a medium sized evergreen tree distributed throughout India particularly in humid areas. *S. asoca* is considered as a sacred tree of Hindus and Buddhists. *S. asoca* has been traditionally used in Indian systems of medicine from time immemorial for treatment of uterine, genital, and other reproductive disorders in females [1]. The earliest chronicled mention of this tree is in the Ayurvedic treatise and later in Charaka Samhita (100 AD) where

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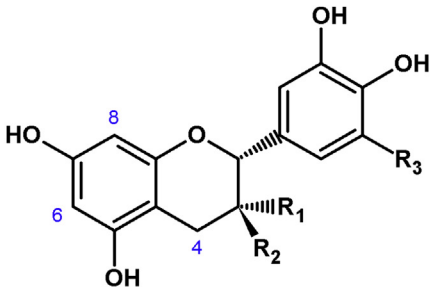
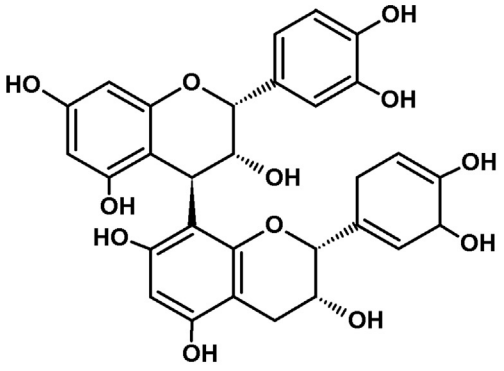
it was recommended as anodynes in formulations for the management of gynecological disorders [2–5]. Bark of *S. asoca* is reported to have stimulating effects on the endometrium, ovarian tissue, and is useful in menorrhagia during uterine fibroids [6]. A number of herbal formulations containing bark (Ashokarishta, Ashokaghrita, etc.) are available in the market. Ashokarishta, a well-known Ayurvedic formulation, is used for the treatment of menstrual disorders. Flowers are also used for the treatment of bleeding piles, cervical adenitis, biliousness, syphilis, hyperdipsia, and hemorrhagic skin diseases.

Phenolic compounds are important for dietary applications and include phenolic acids, polyphenols and flavonoids [7]. Flavonoids are a group of more than 4000 polyphenolics. Beneficial health effects of flavonoids are implicated because of their antioxidant properties and inhibitory role in the processes of carcinogenesis [8]. Catechins and anthocyanins, the glycosides of anthocyanidins contribute to a sizable proportion of total flavonoid consumption by humans. Catechins and anthocyanidins are biogenetically derived from a common C-15 tetrahydroxychalcone precursor, naringenin [9]. Catechins are well known flavonoids used for the symptomatic treatment (relieve the symptoms without addressing the basic cause of the disease) of several gastrointestinal, respiratory, and vascular diseases [10]. *S. asoca* contains significant amounts of phenolic compounds that are considered to be biologically active. A number of compounds including (+)-catechin (CA), (−)-epicatechin (EPC), and (−)-epigallocatechin were reported from *S. asoca*.

Due to variation in the concentration of secondary metabolites, various parts of *S. asoca* have different therapeutic values. Several studies are reported for the determination of individual compounds in different sources and formulations of *S. asoca* by high performance thin layer chromatography (HPTLC) [11–13], high performance liquid chromatography-diode array detection (HPLC-DAD) [14], ultra performance liquid chromatography-quadrupole-time-of-flight mass spectrometer (UPLC-QTOFMS) [15], high performance liquid chromatography-quadrupole-time-of-flight mass spectrometer (HPLC-QTOFMS) [8,16] but there is no report on simultaneous identification and quantification of gallic acid (GA), CA, procyanidin-B2 (PB2), EPC, (−)-epigallocatechin gallate (EGCG), and (−)-epicatechin gallate (EG) (Figure 1) in different parts (barks, flowers, leaves, stems, pods, seeds, and roots) of *S. asoca*. Earlier, Ketkar et al [17] reported an RP-HPLC-DAD method for analysis of GA, CA, and EPC in bark samples of *S. asoca*. However, the reported RP-HPLC-DAD method was not validated. Therefore, it was of paramount interest to study the distribution of polyphenols in different parts of *S. asoca*. In continuation to our earlier work for extraction optimization and profiling of main bioactive constituents of Indian medicinal plants, the principal objectives of the present studies were: (1) to optimize the extraction solvent for maximum recovery of main phenolics; and (2) to develop a simple, selective, precise, and reproducible ultra-performance liquid chromatographic (UPLC) method with a wide linear range and good sensitivity using photo diode array detection for identification and quantification of GA, CA, PB2, EPC, EGCG, and EG in different parts of *S. asoca*.



R_1=OH, R_2, R_3=H; Epicatechin
R_1, R_3=H, R_2=OH, Catechin
R_1=gallic acid ester, R_2, R_3=H; Epicatechin gallate
R_1=gallic acid ester, R_2=H, R_3=OH; Epigallocatechin gallate



Procyanidin B2

Figure 1 – Structure of catechins.

2. Experimental

2.1. *Plant material*

Different parts of *S. asoca* (barks, flowers, leaves, stems, pods, seeds, and roots) were collected from mature trees in the year 2013–2014. The collected plant materials were authenticated by a taxonomist. The specimen samples were deposited in a herbarium. All plant materials were air dried in shade for 1 week. Fine powder of dried samples was made using an electric grinder.

2.2. *Reference compounds and chemicals*

Reference compounds of the highest grade (purity > 99.0 %) namely CA {$[\alpha]_D^{20} = +26 \pm 2$, c = 1, water} was purchased from Natural Remedies (Bangalore, India), while GA, PB2{$[\alpha]_D = 29.2$}, EPC {$[\alpha]_D^{20} = -54$, c = 1, acetone:water (1:1)}, EGCG {$[\alpha]_D^{20} = -188$, c = 1, methanol}, and EG {$[\alpha]_D^{20} = -175.5$, c = 1, ethanol} were purchased from Sigma-Aldrich (Mumbai, India). Methanol, acetonitrile, and phosphoric acid (Merck, Mumbai, India) were HPLC grade. Milli Q grade water used throughout the experiment was prepared using a Millipore purification system (Millipore, Milli Q gradient A10, Molsheim, France).