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Quantification of phenolic content from stem-bark and root of *Hugonia mystax* Linn. using RP-HPLC

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KEYWORDS

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Abstract *Hugonia mystax* Linn. a woody evergreen plant locally known as *Modirakanni* has been used in primary health care by tribals from Tiruvannamalai hills, Tamil Nadu, India. Ethnobotanically, the plant parts are used for rheumatism, skin diseases and inflammation. However, there is no data on active phytoconstituents in stem-bark and root mainly contributing to biological activities. In the present study an attempt has been made to quantify plant phenolics from aqueous, ethanolic and methanolic extracts of stem-bark and root of *H. mystax*. The extracts were also evaluated for their free radical scavenging potential. Quantitative determination of total phenolic content, total flavonoid content and DPPH free radical scavenging activity of plant extracts were carried out using colorimetric methods. Quantitative determination of individual phenolic compounds such as gallic acid, catechol, caffeic acid, vanillin, *p*-coumaric acid and ferulic acid in stem-bark and roots extracts were analyzed using RP-HPLC. The highest phenolic content was found in ethanol extract of root (262.2 ± 0.96 μg of gallic acid equivalent (GAE)/mg of dry plant material), whereas, the highest amount of flavonoids content was found in aqueous extract of root (18.06 ± 1.25 μg of quercetin equivalent (QE)/mg of dry plant material). Q. The highest amount of phenolic acid present was *p*-coumaric acid (3.775 mg/g of dry plant material) in methanol extract of stem-bark. All the solvent extracts of stem-bark and root have shown the presence of *p*-coumaric acid. Methanol extracts of stem-bark and root with IC_{50} values of 175.48 ± 2.14 μg/ml and 169.15 ± 1.10 μg/ml respectively, show potent free radical scavenging activity. In conclusion it can be said that, the plant is rich in phenolics and the major component *p*-coumaric acid may

Abbreviations: RP-HPLC reverse phase, high performance liquid chromatography; TPC, total phenolic content; TFC, total flavonoid content

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probably be responsible for the traditional claims of its biological activities. However, the mechanism of action of the active plant extracts needs to be investigated at the molecular level.

1. Introduction

According to the World Bank report, 80% of the South Asian population still uses traditional plant-based medicines to maintain and improve their health. The World Health Organization listed 21,000 plants used in traditional medicine around the world (WHO, 2002), whereas Schippmann and co-workers estimated this number to be 52,885 (Schippmann et al., 2002). Although there are conflicting claims on the number of plants, it is well established that medicinal plants are routinely used for primary health care in many parts of the world (Pan et al., 2014).

Plants have been integral part of herbal healing processes, which is deep rooted in the systematic classical system of Ayurveda, Siddha, and Unani, as an official health care system (Balakumar et al., 2011; Mohamed Saleem et al., 2011; Pour and Sasidharan, 2011). Folk medicine or oral tradition of health care, which exists among most tribal and rural communities also use medicinal plant knowledge of traditional healers, which is widely recognized as a valid alternative system of medicine (Al-Daihan et al., 2013; Alabri et al., 2014). In developing countries the efforts to recognize and promote the uncodified folk system of medicinal knowledge are still inadequate (Shukla and Gardner, 2006). Systematic scientific investigations are required to prove the traditional claims of effectiveness of these plants and the rationale behind their biological activities needs to be established.

Hugonia mystax Linn. a woody evergreen species, belongs to Linaceae family, which comprise about 40 species in the world; of which *H. mystax* L. was reported from India (Santapau and Henry, 1983; Pullaiah and Chennaiah, 1997). This plant locally known as Modirakanni plays a vital role in the primary health care of tribals from Tiruvannamalai hills, Tamil Nadu, India. The plant parts such as leaves, fruits, bark and roots are extensively used by traditional healers for the treatment of various ailments. Ethnobotanically, leaves and fruits have been used as an antihelmintics and for rheumatism (Sutha et al., 2009; Padel et al., 2010). Roots were used as antihelmintic and also used for dysentery, snake bite, fever, inflammation and rheumatism. Biological activities such as analgesic, anti-inflammatory and ulcerogenic were also reported (Balasubramaniam et al., 1997; Guha Bakshi et al., 2001; Rastogi et al., 2002). The decoction of bark in combination with *Curcuma aromatica*, is given with honey for inflammation in the stomach, vomiting, stomach pain, indigestion (Pushpangadan and Atal, 1984). Roots of *H. mystax* were evaluated for preliminary phytochemical screening and antimicrobial activity. Preliminary phytochemical screening showed the presence of various classes of secondary metabolites such as flavonoids, phenols, saponins, steroids, tannins and terpenoids. Antimicrobial activity of petroleum ether, chloroform, ethanol and aqueous extracts of root showed significant activity against various human pathogens (Vimalavady et al., 2012). This species was reported by Dalzell and Gibson in Bombay Flora in 1861. The species from this geographical location is yet to be explored for its phytochemical constituents and biological activity.

Considering the medicinal importance of the plant and previous findings in our laboratory, an attempt has been made to quantify plant phenolics from stem-bark and roots of the *H. mystax*, extracted with solvents of different polarity.

2. Materials and methods

2.1. Reagents and chemicals

Gallic acid, quercetin, cathechol, caffeic acid, vanillin, *p*-coumaric acid, ferulic acid and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) were purchased from Sigma Chemical Co. (USA). HPLC grade ethanol (EtOH), methanol (MeOH), acetic acid, Folin Ciocalteu's Phenol reagent, sodium carbonate and aluminum chloride were purchased from Merck (Germany).

2.2. Collection of plant material

The stem-bark and root of *H. mystax* (Fig. 1) were collected from Mochemad–Vengurla, Sindhudurg district, Maharashtra. The geographical location of the collection area was 15° 48. 040′ N, 073° 39. 283′ E. The plant specimen was authenticated from the Blatter Herbarium (BLAT), St. Xavier's College, Mumbai. The submitted plant specimen matches with Blatter Herbarium specimen No. 22319 of H. Santapau.

2.3. Preparation of plant extracts

The air-dried stem-bark and roots of *H. mystax* were made into fine powder. 5 g of powder of each plant part were suspended in 50 ml of three different extracting solvent systems

Figure 1 *Hugonia mystax* plant used for the study.