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HPTLC analysis of piperine from *Piper nigrum*, a possible candidate for vititligo treatment

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ABSTRACT

A simple, sensitive and precise high-performance thin-layer chromatographic (HPTLC) method of analysis of piperine in lyophilized dried fruit extracts of Piper nigrum was developed and validated. The separation was carried out on a TLC aluminium plates precoated with silica gel 60F-254 as the stationary phase, eluted with benzene- ethyle acetate (8 : 4) as mobile phase. Densitometric analysis of piperine was carried out in the absorbance mode at 354 nm. This system was found to give compact spot for piperine (Rf value of 0.51). A good linear regression relationship between peak areas and the concentrations was obtained over the range of 15m g/ spot with correlation coefficient 0.99910 ± 2.73. The limit of detection and quantification was found to be 113.71 and 2.14%. The method was validated for precision and recovery. The proposed developed HPTLC method can be applied for identification and quantitative determination of piperine in Piper nigrum extracts.

INTRODUCTION

Dried fruit of *Piper nigrum* (Linnaeus), commonly known as black pepper corn, used in cooking as condiment, is also an important component of many Ayurvedic treatments including those for skin conditions along with piperine, being its main active ingredient which has been studied for properties such as anti epileptic, anti-fungal, abortfacient, sedative hypnotic and muscular relaxant Kapoor, (1990), Johri & Zutshi, (1992) and Pei, (2007). Recently we have found it to be melanogenic also (Sajid and Ali, 2011).

Donata *et al.,* (1990) have used *P. nigrum* fruits orally with other ayurvedic herbs including *Psoralea corylifolia* for the treatment of vitiligo where majority of subjects showed positive response. Despite the use of *P. nigrum* in several ailments cited above, there are no studies on its use as melanogenic agent, except for the work of Lin *et al.,* (1999a) who reported that *P. nigrum* dried fruits and its pure active ingredient piperine induced melanogenesis in cultured mammalian melanocytes. Piperine is a major alkaloid present in black pepper, its IUPAC name is 1-[5-(1, 3-benzodioxol- 5-yl)-1-oxo-2, 4-pentadienyl] piperidine (Trease & Evans, 1983). Piperine provides protection against seizures in epilepsy and has been gaining increasing attention as a bioavailability enhancer in the formulations of several drugs (Timmers, 1994; Karan *et al.,* 1999). It also exhibits a potent chemo-protective effect against procarcinogens and also bacteriostatic, fungistatic, and insecticidal activities (Reen and Rashmet, 1997). A hot-water extract of *P. nigrum* fruit and piperine to stimulate melanocyte proliferation *in-vitro*, and of piperine to stimulate pigmentation *in-vivo* (Lin *et al.,* 1999a). Piperine, because of its protective effect against radiation, can also be administered to cancer patients before radiotherapy (Sharma *et al.,* 2000). Because of the significant pharmacological activities exhibited by the piperine, several researchers have focused on the development of various analytical methods to determine piperine in different matrices such as plant extracts and serum. These methods include spectrometry (Prasad *et al.,* 1997) high-performance liquid chromatography (HPLC) based on UV absorption (Funk *et al.,* 2006). Recently, high-performance thin-layer chromatography (HPTLC) has become a routine analytical technique due to its advantages of its reliability and cost effectiveness (Gantiat *et al.,* 2011).

We have also recently shown that the piperine extracted from *Piperine nigrum* and characterized using HPTLC techniques is a powerful melanogenic active ingredient and can be used as a possible candidate for the treatment of vitiligo, as it had induced powerful melanin dispersion via piperine like receptors in the isolated tail melanophores of *Rana tigerina*, (Sajid and Ali, 2011).