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Ultrasound Assisted Extraction: A Quick and Efficient Method for Extraction of Gymnemagenin from *Gymnema sylvestre* (Retz.) R.Br. ex Sm.

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Abstract

Ultrasound assisted extraction is one of the promising green techniques for extraction of bioactive molecules from medicinal plants, however limited reports are available regarding most of the Indian medicinal plants. Present experiment was carried out to explore the possibility of using ultrasound-assisted extraction as a tool to extract gymnemagenin from an important anti-diabetic medicinal plant, *Gymnema sylvestre*. Mature leaves were shade dried, powdered and extracted using ultrasound processor at 30% amplitude. Extraction was carried out using three levels of hydro-alcoholic solvent (25, 50 and 75%) for five durations (20, 30, 40, 50 and 60 min). Treatments were compared with 24 h cold percolation as control.The extracts were hydrolyzed prior to RP- HPLC analysis in order to convert the gymnemic acids into gymnemagenin. The HPLC analysis of the samples revealed significant variations with respect to gymnemagenin recovery amongst the treatments and ultrasound assisted extraction was found to be faster and more efficient technique for extraction.

Key words: *HPLC, madhunashini, medicinal plant, solvent*

Introduction

Gymnema sylvestre (Retz.) R.Br. ex Sm. is an important medicinal plant of the Apocynaceae family. The species is popularly known as *madhunashini* or *gurmar* owing to its anti-diabetic properties (Dhanani et al., 2015). A number of bioactive macromolecules have been isolated and reported from the leaves of this species (Dateo and Long, 1973; Khramov et al., 2008; Yoshikawa et al., 1991). Gymnemagenin is a common aglycone of gymnemic acids produced after hydrolysis, which is known to inhibit the glucose absorption in the body. This makes the species valuable in the treatment of diabetes in the ayurvedic medicines and gymnemagenin is regarded as a marker compound (Puratchimani and Jha, 2004).

There are a variety of methods used for bioactive molecules extraction from medicinal plants in order to maximize recovery. Solvent extraction is one of the most popular methods in which the solvent enters the solid matrix (generally powder), dissolves the extractable material (solute) and diffuses out the solutes out of the matrix (Zhang et al., 2018). The extracted solutes are then collected and quantified using appropriate methods. In order to recover bioactive compounds from medicinal plants, different solvent extraction methods have been used, including soxhlet extraction, cold percolation, microwave assisted extraction, ultrasound assisted extraction *etc*. Effectiveness of the extraction process is known to vary greatly with a number of factors including the solvent type and amount, method, time, particle size, genotype used, growing conditions, season of cultivation/ collection *etc*. (Li et al., 2008; Zhang et al., 2018).

Ultrasound assisted extraction (UAE) is one of the promising methods, which has been found to be successful in a number of plant species (Barba et al., 2016). The present study was undertaken with the objective to investigate the effect of solvent concentration and extraction time on gymnemagenin recovery from the leaves of *G. sylvestre*.

Materials and methods

Leaf samples were collected from the experimental block of *Gymnema sylvestre* maintained at the ICAR-Directorate of Medicinal and Aromatic Plants, Boriavi, Anand, Gujarat. Mature leaves were shade dried and powdered in a blender. Accurately weighed (5 g) powder samples were extracted with 100 ml of methanol at different solvent compositions and times (Table 1). Briefly, the extraction was performed using three levels of hydro-alcoholic solvent (25, 50, and 75%) for five different time durations (20, 30, 40, 50, and 60 min.) using Sonic Vibra Cell ultrasound processor (VCX 500 Watt) at 30% amplitude. The cold percolation method was also used in the study, using the same amount of powder with three different solvent compositions for a period of 24 h. After extraction, it was filtered under vacuum and concentrated to dryness using vacuum rotary evaporator at 60 °C. Extracts were stored in glass bottles at 4 ºC until further analysis.

Table 1. Effect of time of extraction and methanol concentration on extract yield and gymnemagenin content in *Gymnema sylvestre*

Treatment	Time (min)	Methanol (%)	Extract yield (%)	Gymnemagenin content in extract (%)	Gymnemagenin content in dry powder (%)
T_1	20	75	24.64 ± 1.12 cd	1.460 ± 0.011 cde	0.365 ± 0.003 ef
T_2	20	50	35.59 ± 2.09 a	1.650 ± 0.029 abc	0.588 ± 0.010 a
T_3	20	25	31.67 ± 2.30 ab	0.995 ± 0.016 g	0.314 ± 0.005 fg
T_4	30	75	21.25 ± 2.27 d	1.765 ± 0.033 a	0.375 ± 0.007 de
T_5	30	50	27.48 ± 2.85 bc	1.540 ± 0.010 bcd	0.423 ± 0.003 cd
T_6	30	25	34.80 ± 1.26 a	1.385 ± 0.005 de	0.482 ± 0.002 b
T_7	40	75	24.29 ± 0.67 cd	0.710 ± 0.000 hij	0.172 ± 0.000 j
T_8	40	50	34.73 ± 0.98 a	0.310 ± 0.007 k	0.105 ± 0.002 k
T_9	40	25	34.12 ± 1.53 a	0.795 ± 0.001 h	0.275 ± 0.000 gh
T_{10}	50	75	27.65 ± 1.53 bc	1.635 ± 0.252 abc	0.455 ± 0.070 bc
T_{11}	50	50	32.07 ± 0.60 ab	1.410 ± 0.001 de	0.453 ± 0.000 bc
T_{12}	50	25	33.83 ± 2.36 a	1.315 ± 0.022 ef	0.445 ± 0.008 bc
T_{13}	60	75	22.18 ± 2.65 d	1.700 ± 0.008 ab	0.375 ± 0.002 de
T_{14}	60	50	35.43 ± 2.15 a	0.590 ± 0.011 ij	0.215 ± 0.004 ij
T_{15}	60	25	30.81 ± 1.76 ab	0.530 ± 0.088 j	0.164 ± 0.027 j
T_{16}	24 h	75	31.71 ± 0.16 ab	0.765 ± 0.036 hi	0.245 ± 0.011 hi
T_{17}	24 h	50	34.60 ± 0.62 a	1.175 ± 0.013 fg	0.405 ± 0.005 cde
T_{18}	24 h	25	34.97 ± 0.45 a	1.165 ± 0.007 fg	0.405 ± 0.002 cde

Values presented are the mean ± standard error of mean. Mean values followed by same lower case letter in a column did not differ significantly at 5% level of significance using least significant difference.

The extracts were hydrolyzed prior to HPLC analysis in order to convert the gymnemic acids into gymnemagenin following the method described earlier (Manika et al., 2013). Briefly, in a flask, 50 mg of extract was suspended in 5 ml of methanol, and 1 ml of KOH was added. The flask was boiled on a water bath for 60 min. After cooling the flask, 0.9 ml of concentrated HCl was added. The mixture was once again refluxed on a water bath for 60 min. and then cooled. The pH of the sample was adjusted between 7.5-8.5 using saturated KOH solution. A final volume was then made up to 20 ml using 50% methanol in water. Samples were centrifuged and the supernatants were passed through syringe filter (0.22 µm). Samples were stored at 4 °C until HPLC analysis.

The samples were analyzed using previously developed HPLC protocol (Dhanani et al., 2015). Yield of gymnemagenin were calculated using the equation obtained from the standard curve developed using standard gymnemagenin at different concentrations. Data was subjected to analysis of variance and mean separation was carried out using Web Agri Stat Package (WASP, v. 2.0., ICAR-CCARI, Ela, Goa, India).

Results and discussion

The extraction of bioactive compounds from plant materials is the crucial first step in the analysis of medicinal plants since the plant matrix is complex in nature. Additionally, the extraction conditions determine the recovery of bioactive compounds in the extracts, which govern the efficacy of the extraction. The present study examined the use of ultrasound assisted solvent extraction to reduce the time needed for cold percolation extraction, *i.e.* 24 h. For this, three solvents compositions i.e. methanol (25, 50 and 75%) in water were used for extraction with five extraction durations (20, 30, 40, 50 and 60 min.). Analysis of the data revealed significant differences among the solvent compositions as well as extraction time (Table 1).

Cold percolation is the commonly employed method for extraction in this species (Dhanani et al., 2015). In the present study, in cold percolation treatments, yields of extract remained statistically similar (31.71% to 34.97%), irrespective of the solvent used for the extraction (Table 1). It means, with the prolonged extraction duration of 24 h, the solvent concentration had minimal role in conventional extraction process. The yields obtained from nine out of 15 UAE were comparable to those obtained from cold percolation treatments. As a result, UAE was efficient with short duration treatments. A 75% methanol solvent concentration in the UAE treatment resulted in significantly lower extract yields than 50 or 25% solvent concentrations in the same extraction period, which indicates that solvent concentrations played a significant role in the UAE extraction process. These results are in accordance with earlier researcher (Chemat et al., 2017), who observed solvent concentration dependent variations in extraction efficiency.

The extracts obtained using different treatments were subjected to HPLC analysis to determine the gymnemagenin contents. Results suggested significant variations among the treatments studied. Gymnemagenin content in the extract varied between 0.310% (T_8 involving 50% methanol for 40 min) and 1.765% (T_4 involving 75% methanol for 30 min). The treatment T_4 remained statistically similar with T_2, T_{10} and T_{13}. It means that, though the extract yield was low in treatments involving 75% methanol, the extraction recoveries were not necessarily affected.

Recovery of bioactive compounds was measured in terms of yield from the leaf powder to take into account the extract yield as well as gymnemagenin content. Results suggested that the highest yield of gymnemagenin (0.588%) was obtained in powdered samples extracted using UAE with 50% methanol and 20 min. duration (T_2), while the recovery was the lowest (0.105%), when UAE was carried out for 40 min. using 50% methanol. Gymnemagenin content in conventional cold percolation treatments remained statistically inferior when compared with T_2. Thus, use of UAE with 50% methanol for 20 min. was found to be optimum for extraction of gymnemagenin from leaf powder of madhunashini. The results are in accordance with earlier report by Jovanović et al. (2017) in *Thymus serpyllum*, who obtained higher polyphenols recovery using UAE with 50% hydroalcoholic solvent.

The process of UAE employs energy generated through ultrasonic waves for extraction of the active

ingredients. Cavitation effect hastens the extraction and diffusion of solutes from solid matrix thereby improving the extraction process (Barba et al., 2016). Further, requirement of low amounts of solvents and quicker extraction have been regarded as the prime characteristics of this method (Chemat et al., 2017). Thus the method has advantage over traditional cold percolation method as also witnessed in the present investigation.

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