Development of quality control parameters for the standardization of gymnema sylvestre

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Objective: To develop a novel qualitative and quantitative technique, which can pave the way for rapid and selective determination of different phytoconstituents of Gymnema sylvestre (G. sylvestre). Methods: Phytochemical test, TLC analysis, solubility study, total phenol and flavonoid content and antimicrobial study were performed in the present investigation. Fingerprint analysis and quantitative analysis of quercetin were also performed through hptlc method. Results: alkaloid, saponin, tannin, triterpenoid and flavonoid were found to be present in the G. sylvestre extract. Solubility in water and alcohal, moisture content and gymnemic acid content were found to be 86.36%, 88.24%, 4.20%, and 26.24% w/w, total phenol and flavonoid content were found to be 0.80% and 1.90%. Microbiological assay showed that E. coli and salmonella were found to be absent whereas total bacterial count and yeast & moulds contents were found to be 650 and 60 cfu/g. quantitative analysis through hptlc revealed the presence of 2.95% w/w of quercetin. Conclusions: in future this study will be helpful for the quantitative analysis of phytoconstituents as well as standardization of the G. sylvestre.

1. Introduction

Gymnema sylvestre (G. sylvestre) (retz.) schult. belonging to family asclepiadaceae is widely distributed in the different parts of the world. G. sylvestre mainly found in the southern China, Vietnam, Deccan peninsula of western India, tropical Africa, Malaysia, Srilanka, Japan, Germany and USA. G. sylvestre is well known for its sweet taste suppressing activity and found to be useful for the treatment of diabetes mellitus and obesity[1–3]. In traditional medicine G. sylvestre is used as a stomachic, diuretic and remedy for diabetes mellitus. water extract of the leaves of G. sylvestre inhibited absorption of glucose in the small intestine, and have inhibitory action against glucaen synthesis by glucosyltransferase. antiurolonic and antiviral effect of G. sylvestre have also been suggested[4,5], the plant extracts are also used in folk, ayurvedic and homeopathic systems of medicine[1]. G. sylvestre has traditional uses in the treatment of asthma, eye complaints and snake bite. it also possesses anti–microbial, anti–hypercholesterolemic and hepatoprotective properties[6], gymnemic acid (ai–a4) and a number of triterpenoid saponins have been isolated from the G. sylvestre plant. they contain not only gymnemagnenin, but also 23–hydroxylongispinogenin, gymnestrogenin and a few dammarane derivatives as the aglycones [4]. a new flavonol glycoside, kaempferol 3–o–b– d–glucopyranosyl–(1f4)–a–l–rhamnopyranosyl–(1f6)– b–d–galactopyranoside, kaempferol 3–o–robinobioside, rutin, quercetin 3–o–robinobioside, and tamarixetin 3–o–robinobioside have been also isolated from G. sylvestre[2], a number of gymnema products such as gymnema capsules, gymnema tea, bioshape®, and diximol® have been developed and used for the treatment of different diseases in the world[1].

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2. Material and methods

2.1. Plant extract, chemicals and reagents

The extracts of *gymnema sylvestre* were procured from garlico herbal concentrate, m. p. india. High performance thin layer chromatography (hptlc) precoated plates (silica gel merck 60f254) was used as a stationary phase. All the other chemical and reagents used in this study are analytical grade.

2.2. Development of standard analytical parameters

Preliminary phytochemical analysis of *G. sylvestre* extract was done according to the standard official methods[7]. Thin layer chromatography (tlc) analysis was done according to the standard protocol[8,9]. solubility, moisture content, gymnemic acid content and microbiological analysis were performed according to the standard official methods[10,11]. Total phenol and flavonoid content of *G. sylvestre* were also determined[12,13]. Further fingerprint analysis and quercetin content of *G. sylvestre* were also determined using hptlc techniques. For the preparation of the calibration curve in the quantitative analysis different concentration of the standard stock solution were prepared in the hplc grade methanol. All the needed concentration of the samples was prepared from the stock solution by suitable dilution. The chromatographic conditions for the hptlc analysis used in the present investigation are as follows.

3. Results

Organoleptic study revealed the presence of greenish yellow colour and bitter test of *G. sylvestre* extract. Alkaloid, saponin, tannin, triterpenoid and flavonoid were found to be present in the *G. sylvestre* extract. Tlc analysis showed the presence of four spots with rf (0.26, 0.32, 0.40, 0.94) in ethyl acetate: methanol: h20 (81:11:8) and rf (0.52, 0.62, 0.75, 0.94) in n–butanol: acetic acid: water (5:1:5) solvent systems respectively. Solubility in water and alcohol were found to be 86.36% and 88.24%. Moisture content and gymnemic acid content were found to be 4.20% and 26.24% in the *G. sylvestre* extract. Total phenol and flavonoid content were also determined and found to be 0.80% and 1.90% in respect to standard gallic acid and rutin. microbiological assay showed that *E. coli* and salmonella was found to be absent whereas total bacterial count and yeast & moulds contents were found to be 650 and 60 cfu/g. optimization of hptlc solvent system was done and ethyl acetate: formic acid: glacial acetic acid: h20 (100:11:11:26) was found to be the most suitable solvent system for fingerprint analysis with the respective rf (0.15, 0.22, 0.64, 0.99) and peak area (7.56%, 6.42%, 9.86%, 76.16%). content of quercetin in *G. sylvestre* was analysed through hptlc methods and was found to be 2.95% w/w.

4. Discussion

Physicochemicals have been used for the treatment and prevention of various health ailments from time immemorial. a large percentage of the drugs prescribed worldwide are derived from plants and 121 such active compounds are in use. Who’s essential medicine list contain large number of drug from plant origin[14]. Physicochemical standards were generally used for deciding the identity, purity and strength of the drug source. These parameters were also used to detect the adulterants if any present in the plant materials[15]. The physicochemical parameters of the drug are an important parameter in detecting adulteration or improper handling of drugs. *G. sylvestre* contain flavonoid, tannin, saponins and alkaloid which have various pharmacological activities such as anti–inflammatory, anti allergic, antioxidant, anti diabetic, anti–viral and many more[16–20].

Phytochemical standardization is one of the tools for the standardization of the herbal drug, which includes preliminary phytochemical analysis and quantification of different phytoconstituents (marker compounds) present in the extract. In hptlc analysis we can analyze several components simultaneously, using a small quantity of marker compound and mobile phase with very less time[21].

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Estimation of quercetin in <em>G. sylvestre</em> extract.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plate material</td>
<td>Hptlc precoated plates silica gel merck 60f254</td>
</tr>
<tr>
<td>Solvent system</td>
<td>Ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26)</td>
</tr>
<tr>
<td>Syringe</td>
<td>100 μL Hamilton (bonadzu, switzerland)</td>
</tr>
<tr>
<td>Application mode</td>
<td>Camag automatic tlc sampler iii</td>
</tr>
<tr>
<td>TLC chamber</td>
<td>Camag, and 2 automatic developing chamber</td>
</tr>
<tr>
<td>Development mode</td>
<td>Ascending</td>
</tr>
<tr>
<td>Scanning</td>
<td>Camag tlc scanner 3 with cats software</td>
</tr>
<tr>
<td>Experimental conditions</td>
<td>Temperature (25±2) °C, relative humidity 40%</td>
</tr>
</tbody>
</table>
hptlc is an inexpensive method for separation, identification, and quantitative analysis of samples and it can be used to solve many qualitative and quantitative analytical problems in a wide range of fields, including medicine, pharmaceuticals, chemistry, biochemistry, food analysis, toxicology and environmental analysis[22].

In conclusion, from the above finding we can interpretate that the G. sylvestre contained considerable amount of phytoconstituents. This analytical method can be utilizes for the determination of phytoconstituents present in the G. sylvestre. In future, these characters are also used to check the genuine nature of the crude drug, thus it plays an important role in preventing the possible steps of adulteration

Conflict of interest statement

The authors report no conflict of interest.

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