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Research article

QUANTITATIVE SPECTROPHOTOMETRIC ESTIMATION OF TOTAL ALKALOIDS IN *WITH ANIA SOMNIFERA* L. IN VIVO AND *IN VITRO*

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ABSTRACT: In this study, plant parts (roots, stem and leaf) and callus of Withania somnifera (family: Solanaceae) commonly called Ashwagandha were analyzed by spectrophotometer for their alkaloid content..A simple spectrophotometric method based on the reaction with bromocresol green (BCG) has been developed for determination of total alkaloids in medicinal plants. A yellow complex forms and is easily extractable by chloroform at pH 4.7(Fazel Shamsa et al, 2008). The absorbance of the complex obeys Beer's law over the concentration range of 4-13 µg atropine per ml of chloroform. This procedure can be carried out in the presence of other compounds without interference.

Key words: Atropine, Bromocresol green, *Withania somnifera*, alkaloids, Spectrophotometer

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INTRODUCTION

*Withania somnifera (l.)*Dunal is commonly known as ashwagandha and belongs to family Solanaceae. All the parts of the plants have shown remarkable significance in the field of pharmacology. The plant contains withanolides A and D.

Ashwagandha withanolides are anti-inflammatory and enhance the body's defense against infections and tumour (Jaffer et al. 1988, Devi and Sharada, 1992). Its roots are prescribed for hiccups, female disorders, cough, rheumatism, dropsy, low energy and arthritis (Trivedi et al. 2015) The roots are also used as sedative for senile debility and for the prevention and inhibition of Alzheimer's disease.(Kirtikar and Basu, 1975). Therefore determination of total alkaloids is very important related to the quality of medicinal plants.

Most of the reported spectrophotometric methods suffer from disadvantages such as narrow range of determination. They require heating or extraction, a long time is needed for the reaction to be completed, and the colored product formed is unstable. The purpose of the current work was to provide a simple, sensitive, and rapid spectrophotometric method for the determination of total alkaloids in medicinal plants. The method is based on the reaction of alkaloid with bromocresol green (BCG), forming a yellow-colored product. The low yield and high market price of the pharmaceutically important alkaloids have created interest in improved alternative routes for their production such as using cell and tissue culture. Therefore, the present study aimed trial to alkaloid production *in vivo* (plant leaves, stems and roots) and *in vitro* callus culture conditions.

MATERIALS AND METHODS

Plant Material

Plants of *Withania somnifera* (Source-Sanjay Gandhi Biological Park, Patna) grown in the pot in the departmental garden of Botany, Patna University were used as an experimental material. Different explants viz, young leaves, root segments and nodal section of the plant were used for establishing calli. The leaves, stems and roots of *W.somnifera* were collected. The plant parts were washed with deionized water, shade dried till the weight become constant at room temperature and ground to fine powder.

Callus induction

For callusing, nodal segments were cultured on MS medium supplemented with 1 mg/l 2-4D + 1.5 mg /l BAP. Leaf showed maximum callus formation on MS medium with IBA at the concentration of 1.5 mg/liter. Callus was compact and greenish yellow colored. Callus obtained after 8 weeks of culture was further evaluated for alkaloid production.

Preparation of solutions

Bromocresol green solution ($1x10^{-4}$) was prepared by heating 69.8 mg bromocresol green with 3 ml of 2N NaOH and 5 ml distilled water until completely dissolved and the solution was diluted to 1000 ml with distilled water. Phosphate buffer solution (pH 4.7) was prepared by adjusting the pH of 2 M sodium phosphate (71.6 g Na2HPO4 in 1 L distilled water) to 4.7 with 0.2 M citric acid (42.02 g citric acid in 1 L distilled water). Atropine standard solution was made by dissolving 1mg pure atropine (Sigma Chemical, Bangalore) in 10 ml distilled water.

Preparation of standard curve

Accurately measure aliquots (0.4, 0.6, 0.8, 1 and 1.2 ml) of atropine standard solution and transfer each to different separatory funnels. Then, add 5 ml pH 4.7 phosphate buffer and 5 ml BCG solution and shake a mixture with 1, 2, 3 and 4 ml of chloroform. The extracts were collected in a 10-ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without atropine (Fig-1).

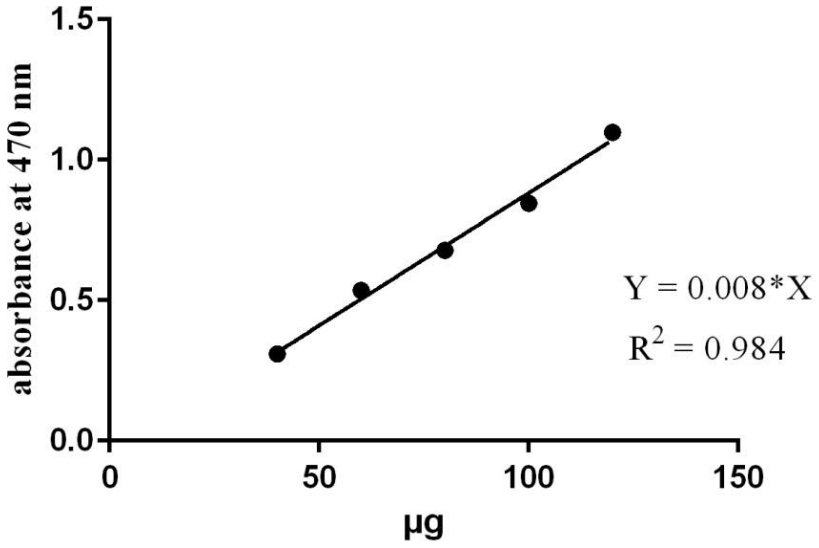

Fig-1: Standard curve

Extraction

The plant materials (root, stem and leaf) and callus (100g) were ground and then extracted with methanol for 24 h in a continuous extraction (soxhlet) apparatus. The extract was filtered and methanol was evaporated on a rotary evaporator under vacuum at a temperature of 45^0C to dryness.

A part of this residue was dissolved in 2 N HCl and then filtered. One ml of this solution was transferred to a separatory funnel and washed with 10 ml chloroform (3 times). The pH of this solution was adjusted to neutral with 0.1 N NaOH. Then 5 ml of BCG solution and 5 ml of phosphate buffer were added to this solution. The mixture was shaken and the complex formed was extracted with 1, 2, 3, and 4 ml chloroform by vigorous shaking. The extracts were collected in a 10-ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm.

RESULTS AND DISCUSSION

A yellow-colored complex with a maximum absorption was developed. This complex was completely extractable by chloroform at pH 4.7. A calibration curve was plotted for various concentrations of atropine (Figure 1).Beer's law was followed over the concentration range of 4-13 µg atropine per mL of chloroform. The effects of temperature and pH were studied. A pH 4.7 gave optimum results and different temperatures had no effect on complex formation and extraction. The complex was very stable in chloroform and began to fade slowly only after 10 days. Before the extraction, the mixture was put in a boiling water bath for 3 min. The absorbance did not change after extraction with chloroform. The plant materials have been extracted by a method that only alkaloids come into the final residue and therefore other organic compounds which react with BCG, do not exist in the final solution (Sakai et al,1991).

Table 1 shows the amount of total alkaloid in tested plant materials determined by BCG-complex formation method.

Table 1: Total alkaloids in various parts of *Withania somnifera*

S.No.	Plant Species	Part Investigated	Total Alkaloids mg CE / 100g
1.	*Withania somnifera(L.) Dunal*	Root	0.0088
2.		Stem	0.0067
3.		Stem Callus	0.0077
4.		Leaf	0.0071
5.		Leaf Callus	0.2032

The results showed that the callus leaf extract showed highest alkaloid content followed by root extact of mother plant. The callus stem sample has also more alkaloid content than stem of mother plant.In in vivo plant parts root showed highest alkaloid content. The alkaloid content was varied with respect to the parts analysed.

These fluctuations in the concentration and quantities of secondary metabolites are basically due to environmental influences (Supe et al., 2011). The callus developed on Murashige and Skoog (MS) media supplemented with different concentrations of auxins and cytokinins was found to have variable alkaloid contents (Verma *et al*. 2012). MS media with 2,4-D and BAP were found to be the most promising media in relation to callus biomass increase and total alkaloid content(Verma et al,2012).

Spectrophotometric determination of total alkaloids with bromocresol green is a simple and sensitive method and does not need very special equipment. The proposed method has the advantage of being less time consuming, with the assay requiring an average of 1 h. The BCG can react with a certain class of alkaloids (alkaloids that have nitrogen inside their structure) and amine or amid alkaloids does not react with this reagent (Amanlou, 2007). Therefore the method described in this study can be used for determination of a special group of alkaloids.

CONCLUSION

The present study illustrates the production of slightly increased concentrations of total alkaloids in the *in vitro* cultured cells when compared to the plant samples. Methanol was found to be a better solvent for the extraction of most of the phytochemicals. Therefore, *in vitro* culturing may be considered as a better method to harvest higher concentrations of bioactive phytochemicals than the stem/leaf samples.

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