

Effect of solvent composition on total phenol and flavonoids content of *Withania somnifera*

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ABSTRACT

Withania somnifera, an herbal medicine is commonly used for rheumatoid arthritis in India. In the present study, total phenolic content and flavonoid content of *Withania somnifera* extracts of two commercially available herbal medicines were investigated by varying the composition of solvent. Water and various concentrations (10%, 50%, 90%, and 100%) of methanol in water were used as solvent in the extraction of *Withania somnifera* samples. The total phenolic content and flavonoid content of *Withania somnifera* extracts were investigated using UV-Visible spectrophotometer. The results show that both phenolic and flavonoid contents are highly dependent on composition of extracting solvent. The extract obtained by 50% methanol showed the highest total phenolic content in the samples ranges from (0.74-1.2 mg gallic acid equivalent/g) and the highest flavonoid content obtained from 100% methanol ranges from (2-2.2mg rutin equivalent/g). These results indicate that *Withania somnifera* is rich source for phenols with a potential to reduce oxidative stress.

KEY WORDS: *Withania somnifera*, Phenols, Flavonoids, rutin, gallic acid.

1. INTRODUCTION

In India, traditional use of herbal medicines implies significant historical use, and this is definitely right for many products that are existing as 'traditional herbal medicines'. In India, a large number of people dependent on traditional medicinal plants for its efficiency, safety and to meet health care needs. Secondary metabolites of medicinal plants have a tremendous use in modern and traditional medicine, and are the sources of important drugs. Secondary metabolites, particularly, the phenolics have gained substantial significance due to their potential health aids. Various studies have shown that herbal plant materials containing antioxidants is effective for lowering the occurrence of various diseases. Recovery of phenolic compounds from plant materials is typically depends upon different extraction techniques, such as solid-to-liquid ratio, solvent composition, time of extraction, particle size of plant materials, temperature and p^H. The polarities of the polyphenols range from polar to non-polar, thus a wide range of solvents and their mixtures for their extraction has been studied. Aqueous mixtures of methanol and ethanol have been extensively used to extract phenolic compounds from various plants.

The herbal medicine selected for the present investigation is *Withania somnifera*, has been used for lowering cortisol and balancing thyroid hormones. It is also used for stress relieving and to enhance stamina, hence it is referred as Indian ginseng. The main objective of the present study was to investigate the effective solvent composition for extracting potent phenolic compounds and flavonoids from commercially available *Withania somnifera* of two different manufacturers.

2. MATERIALS AND METHODS

Chemicals: Analytical grade methanol, sodium nitrite, sodium carbonate, Folin–Ciocalteu reagent, rutin, gallic acid and aluminium chloride were purchased and used in the present study.

Sample preparation: *Withania somnifera* of two different manufacturers were purchased from the local market, Andhra Pradesh, India in tablet form. The samples were ground to powder and sieved to obtain fine particles. One gram of each sample was macerated with 50 ml of 100%, 90%, 50%, 10% of methanol and 100% water separately for 48 h with occasional shaking and the filtrate through wattman No.42 filter paper. The extracts were analyzed for total phenolics and flavonoids content.

Determination of Total flavonoid content: To one ml of sample extract 0.5 mL of 10% AlCl₃ and 0.5 ml of 5% NaNO₂ was added and diluted to 10 mL with distilled water. Absorbance of the reaction mixture was read at 420 nm after 30 min of incubation. The amount of total phenolic compound in the sample extract was determined from the standard rutin curve equation $y = 0.106x - 0.013$; $R^2 = 0.994$. Total flavonoid content of the sample extract was expressed as rutin equivalent per gram of sample. All samples were analyzed in triplicates.

Determination of total phenolic content: Total phenolic content was determined by Folin-Ciocalteu reagent method as described by Pourmorad et al. To one ml of sample extract 0.5 mL of 20% FC reagent and 2 ml of 20% sodium

carbonate was added and diluted to 10 mL with distilled water. After 30 min of incubation at room temperature the absorbance against the blank was determined at 760 nm. The amount of total phenolic compound in the sample extract was determined from the standard gallic acid curve equation $y = 0.106x - 0.013$; $R^2 = 0.994$. The total phenolic content of the plant was expressed as gallic acid equivalent (GAE)/g of sample. All samples were analyzed in triplicates.

3. RESULTS AND DISCUSSION

The concentration of flavonoids and phenolics of *Withania somnifera* of manufacturer A and B samples at different compositions of solvent are presented in the figures.1 and 2. From the tables it is clear that the total phenol and flavonoids contents of *Withania somnifera* extracts are highly dependent on the composition of the solvent.

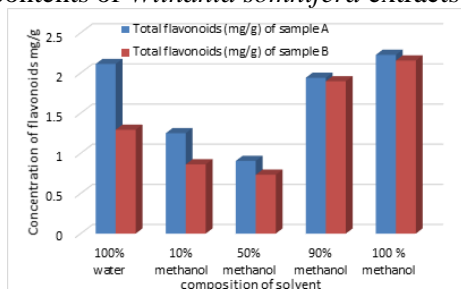


Figure.1. Total flavonoids content

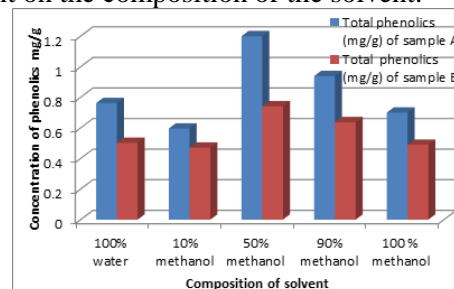


Figure.2. Total phenolics content

The amounts of total flavonoids extracted from *Withania somnifera* samples using different solvent composition were ranged from 0.73-2.22 mg RE/g, showing considerable variations among different extracts tested in this study (Table.1). Using pure methanol we were able to extract the higher amounts of flavonoids (sample A: 2.22 and sample B: 2.15 mg RE/g), whereas much lower amounts were extracted with 50% methanol (sample A: 0.73 and sample B: 0.90 mg RE/g). The flavonoids content of two different manufacturers A and B is in close agreement with the solvent composition. The total phenolic content values of the extracts range from 0.47-0.93 mg GE/g (Table.2). 50 % aqueous methanol contains higher total phenolic compounds (sample A: 1.2 mg GAE/g and sample B: 0.74 mg GAE/g). 10% methanol extracts contains lower amounts of phenolic compounds. Moreover, the total phenolic content of the 100% methanol extract is good agreement with the 100% water extract.

Table.1. Total flavonoids concentrations of *Withania somnifera* samples A and B

Solvent composition	Total flavonoids (mg/g) of sample A	Total flavonoids (mg/g) of sample B
100% water	2.1120	1.2931
10% methanol	1.2500	0.8620
50% methanol	0.9051	0.7327
90% methanol	1.9396	1.8965
100 % methanol	2.2258	2.1551

Table.2. Total phenolics concentrations of *Withania somnifera* samples A and B

Solvent composition	Total phenolics (mg/g) of sample A	Total phenolics (mg/g) of sample B
100% water	0.7625	0.5020
10% methanol	0.5958	0.4708
50% methanol	1.2000	0.7416
90% methanol	0.9395	0.6375
100 % methanol	0.7000	0.4895

4. CONCLUSION

Total phenol and flavonoids contents of herbal extracts are highly dependent on the composition of the solvent. Based upon the results of the present investigation it can be concluded that 50% aqueous methanol is most effective solvent for recovering phenolic components from *Withania somnifera* while the 100% methanol and water is most effective for flavonoids.

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