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## Quantifying phytophenols in *Andrographis paniculata* and *Withania somnifera* leaf extracts

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### ABSTRACT

*Andrographis paniculata* (Nilavembu) and *Withania somnifera* (Ashwagandha) are some of the commonly available herbs, used for treating a wide range of ailments in man and animals. Both the plants possess a wide spectrum of pharmacological effects attributed to their various active phytochemical constituents. The present study was undertaken to assess the qualitative phytochemistry and to estimate the total phenolic content of leaf extracts of the two plants by Spectrophotometry and Thin Layer Chromatography. The results thus obtained suggest that the leaves of *Andrographis paniculata* and *Withania somnifera* are potential sources of healthy phytochemicals especially phenols.

**Keywords:** phenolics, thin layer chromatography, spectrophotometry.

### INTRODUCTION

Herbs have been used traditionally by native healers to treat many common ailments of human and livestock. Recent developments in the field of phytomedicine research have emphasized more on the scientific evaluation of medicinal herbs against usual drugs owing to their immense beneficial properties. As medicinal plants produce several phytochemical compounds they can be suitably used as an alternative source of medicine to synthetic drugs. Phytochemicals play important roles in plant defence mechanisms and are mainly classified into carotenoids and polyphenols [1].

*Andrographis paniculata*, known as the King of Bitters, is an annual profusely branching herb in the family Acanthaceae. It is extremely bitter in taste. The whole plant, mainly the roots and leaves are known for their healing properties and have been used extensively in Siddha and Ayurveda. Andrographolide, a bicyclic diterpenoid, is a major component that is extracted from the leaves of this plant. Other constituents include paniculide, panicoline, andrographine and methoxyflavone [2, 3].

*Withania somnifera*, popularly known as the Indian Ginseng, is an important herb known for its health promoting effects. It is said to calm the mind by creating a sense of well being in the individual, relieving weakness and nervous exhaustion and also by promoting a healthy sleep. Hence this herb is termed as rasayana [4]. Anolides, a group of steroidal lactones, isolated from the leaves of this plant, are responsible for the pharmacological properties they possess [5].

In the present study, the qualitative phytochemistry of *Andrographis paniculata* and *Withania somnifera* was assessed and their total phenolic contents were estimated by Spectrophotometry and Thin Layer Chromatography.

### MATERIALS AND METHODS

#### Plant Source

Fresh leaves of *Andrographis paniculata* (AP) and *Withania somnifera* (WS) were collected from Herbal Garden, Ethno Veterinary Herbal Product Research and Development Centre, Tamil Nadu Veterinary and Animal Sciences University, Orathanadu, Thanjavur District, Tamil Nadu. The leaves were cleaned, shade-dried and finely powdered for further analysis.

#### Preparation of Methanolic Extracts

25g of the powdered sample was extracted with 125ml of methanol for 6 hours in a Soxhlet Apparatus [6]. The extracts were kept in a desiccator allowing the solvent to evaporate completely.

### Preliminary Phytochemical Screening

As per standard protocols, leaf extracts of the two plants were screened for the presence of various phytochemical compounds.

### Thin Layer Chromatographic Analysis

TLC was carried out on a 20x20 cm silica gel pre-coated aluminium sheets (Merck). 10 µl of each plant extract was placed on the TLC plate at an equal distance. The plate was then kept immersed in chloroform:ethanol (7:3) solvent system in a chromatographic chamber [7]. After 10 minutes, it was subjected to Iodine vapour in a glass beaker containing iodine crystals [8]. A change in the colour of the sample-run spots indicates the presence of phenols. The Retardation factor (Rf) which signifies the ratio of distance travelled by a compound to that of a solvent in a given time, was also measured for the sample extracts.

### Phytophenolic Estimation

The total phenolic content (TPC) of the two leaf extracts was estimated by Folin-Ciocalteu reagent method [9]. A standard calibration curve was plotted with Gallic acid solutions ranging from 2-10 µg/ml concentrations [10]. To 100 µl of each leaf extract, 750 µl of Folin-Ciocalteu reagent was added and shaken well for 5 minutes. To this, 750 µl of 6% sodium carbonate was added and the mixture was incubated for 90 minutes at room temperature. Absorbance was read at 765 nm in a UV-Spectrophotometer. TPC was determined using an equation obtained from the calibration curve and expressed in terms of gallic acid equivalent (GAE) [11, 12].

## RESULTS AND DISCUSSION

The results of the preliminary phytochemical screening of the two methanolic leaf extracts are summarized in Table 1. *Andrographis paniculata* showed the presence of alkaloids, glycosides, phenols, flavonoids, terpenoids and saponins similar to earlier reports [13, 14]. Among the various metabolites in AP, the most notable are the terpenoids which account for a large proportion, apart from flavonoids, xanthenes, polyphenols, macro and micro elements [15]. Phytophenols are the main antioxidants that effectively scavenge free radicals which are abundantly distributed in plants [16]. However, a variability in chemical contents of different plant parts of AP has been attributed to different factors like geographical area and agricultural practices followed [17, 18]. On the other hand, *Withania somnifera* showed the presence of alkaloids, glycosides, phenols, flavonoids, saponins and terpenoids similar to previous studies [19, 20]. An earlier report has shown the presence of alkaloids, steroidal compounds, saponins, starch, sugars and a variety of amino acids [21, 22]. WS has exposed chemogenetic variation with three chemotypes I, II and III reported so far. These are structurally similar but differ in their chemical constituents [23].

**Table 1:** Preliminary Phytochemical Screening of methanolic leaf extracts

Phytochemical compound	Method	<i>Andrographis paniculata</i>	<i>Withania Somnifera</i>
Alkaloids	Dragendorff test	+	+
Glycosides	Keller Killani Test	+	+
Tannins	Ferric chloride test	-	-
Saponins	NaOH test	+	+
Phenolics	Ferric chloride test	+	+
Flavonoids	Shinoda Test	+	+
Terpenoids	Noller Test	+	+
Steroids	Salkowski Test	-	-

The results of the estimation of total phenolic content by spectrophotometry are presented in Table 2. *Andrographis paniculata* contained 30.99 mg GAE/g sample of TPC. Conversely, AP sourced from Malaysia and different parts of India contained variable concentrations of total phenolics [24-26]. This variability was seen with phenolics determined using different solvents based on the chemical reducing capacity of the extracts related to gallic acid [27]. *Withania somnifera* was found to contain 232.44 mg GAE/g sample of TPC. A nearly similar report showed WS having 308 mg GAE/ g sample [28].

**Table 2:** Estimation of Total Phenolic content by Spectrophotometry

Plant	TPC (mg GAE/g of sample)
<i>Andrographis paniculata</i>	30.99
<i>Withania somnifera</i>	232.44

The results of Thin Layer Chromatographic analysis are presented in Table 3 and Figure. 1. TLC developed in the mobile phase of chloroform: methanol: acetic acid for methanolic extracts of *A. paniculata* and *W. somnifera* with gallic acid as the reference compound showed Rf values of 0.65 and 0.67 respectively, compared to the gallic acid standard of 0.7, after exposure to iodine vapour, which is in agreement with many previous results [29, 30]. The TLC of isolated AP samples carried out in different solvent systems showed Rf values varying from 0.2 to 0.9. This could determine the purity of the active principles present in AP [31, 32]. While one report showed a nearly similar Rf value of WS to that of the present study, another study showed that variation in the TLC pattern is speculated to be due to the formation of newer intermediate compounds during the course of development of some of the major phytochemical compounds such as withanolides and bacosides [33, 34].

**Table 3:** Thin Layer Chromatographic Analysis

Plant	Retardation factor (Rf)
<i>Andrographis paniculata</i>	0.65
<i>Withania somnifera</i>	0.67
Gallic acid standard	0.70



**Figure 1:** TLC plate showing the presence of phenols

## CONCLUSION

*Andrographis paniculata* and *Withania somnifera* are rich in healthy phytochemical compounds, essentially the phenolics. The presence of polyphenols in Nilavembu and Ashwagandha can confer to their significant antioxidant properties which is valuable for treating several common ailments. Although, inclusion of these plants in human or ethno-veterinary formulations can contribute to several health benefits in man, livestock and poultry; more emphasis on further research is suggested to highlight their other potential advantages.

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