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A Study on Phytochemical Screening and Analysis of Leaf Extract of *Withania Somnifera*

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Abstract*—Withania somnifera* is a highly renowned plant in the Indian Ayurvedic and Unani System of medicine. This plant is known to promote physical and mental health and is used to treat almost all of the disorders that affect human health. So, it is called as' Power Herb'. The present study investigated the qualitative and quantitative phytochemical analysis of *W. somnifera*. The leaves were shade dried and powdered and sequentially extracted with Hexane, Ethyl acetate, and Ethanol solvents. Qualitative and Quantitative Phytochemical analysis was performed for the three extracts. The phytochemical analysis showed the presence of carbohydrates, tannins, saponins, flavonoids, alkaloids, quinones, phenols, etc., Quantitative estimation of the phytochemicals was also evaluated. Biologically active phytochemicals were mainly present in the Ethyl acetate extract of *W. somnifera*.

***Keywords* —** Amukkura, Extraction, Phytochemical analysis, Tannins, Flavonoids

I. INTRODUCTION

India is one of the few countries wherever most of the familiar medicinal plants may be cultivated in many parts of the country. The ancient medicinal system is mainly dependent on medicinal plants that allow using most of our indigenous plants. India has approximately 2000 species of medicinal plants and has a significant geographic region with varying agro-climate conditions of high production potential. India is now a major exporter of medicinal plants, Rs 86 crores worth of raw materials & medicinal plant drugs are expected to be exported from India. Medicinal Plants are rich in secondary metabolites and they are potential drug sources. *Withania somnifera (L.)* Dunal belongs to the family Solanaceae . *W. somnifera* is considered as a wonder drug and power drug in the Indian medical system and it is extensively used because of its unique properties. This plant is usually known as Ashwagandha, Amukkura, Indian ginseng, poison gooseberry or winter cherry. This plant has been categorized as a Rasayana drug. Rasayanas are used to encourage physical and mental health by enhancing the resistance against diseases and adverse environmental influences, to arrest the aging cycle, to revitalize the body under stressed conditions, and as a mood calming nervine tonic (Rege *et aI*., 1999) [1].

The therapeutic activities of *W. Somnifera* include anti-osteoporotic, anti-arthritic, anti-hyperglycemic, neuro-pharmacological, cardioprotective, immunomodulatory, musculotropic, radiosensitizing, hepatoprotective, chemoprotective, anti-aging, macrophage-activating, diuretic, hypocholesterolemic, aphrodisiac, rejuvenating, and hemopoietic (Pandey *et al.,* 2018) [2] as well as recovery from neurodegenerative disorders (Parul Gupta, *et al.,* 2016) [3]. Despite of its immense health benefits, the *W. somnifera* has become extinct from its natural habitat and is now one of the endangered species [4].

II. RELATED WORK

Due to substances known as phytochemicals, many plants have medicinal properties. Numerous techniques, such as methods of extraction, separation and analysis and toxicology and bioactivity tests, must be planned and implemented to use these plants in operation. In Ayurveda, *W. somnifera*, leaves and roots are used to treat inflammation-related disorders. This plant has been extensively researched for its biologically active components, steroidal lactones and withanolides. Because of its tremendous significance, this plant was selected in this present study to evaluate its phytochemical activities. Therefore, the aim of this study to identifying qualitative and quantitative phytochemistry from the leaf extract of *W. somnifera*. Phytochemical studies help to standardize herbal preparation and to understand the importance of phytoconstituents in terms of their medical uses.

III. METHODOLOGY

Collection and Authentication of Plant Material

The plant samples were collected from Pannapatti of Salem district in Tamil Nadu. The leaves were gently washed with tap water and then rinsed with distilled water. The plant specimen was identified and authenticated by Plant Anatomy Research Centre, Chennai, India with voucher specimen no. (PARC/2019/4091). The collected samples were cleaned from impurities and shade dried and kept away from sunlight to prevent changes in the nature of the plant constituents. After drying, the leaf samples were pulverized to a coarse powder using a mechanical grinder and stored in an airtight container for further studies.

Extraction and Fractionation of Plant Material

Successive sequential Extraction

About 1 kg of shade-dried powdered plant material was extracted using soxhlet apparatus successively with different solvents such as hexane, ethyl acetate, and ethanol in the order of increasing polarity. Each time before extracting with the next solvent, the marc was dried. The extracts were then concentrated by evaporating the solvent using a rotary vacuum evaporator. The extract obtained with each solvent was weighed and the percentage yield was calculated. The extracts were kept in bottles and were used for further phytochemical and biological screening studies.

Qualitative Phytochemical Analysis

The hexane, ethyl acetate and ethanol extracts were subjected to qualitative chemical analysis. The plant extracts were qualitatively analyzed for the presence of different phytochemical metabolites such as carbohydrates, tannins, alkaloids, flavonoids, saponins, glycosides, cardiac glycosides, terpenoids, triterpenoids, phenols, coumarins, anthraquinones, Phlobatannins, steroids and phytosterols by standard protocols described by Trease and Evans *et al.*, 1983 [5] and Sofowora and Harborne *et al.*, 1973. [6-7].

a. Test for carbohydrates A few drops concentrated sulphuric acid and 1 ml of Molisch 's reagent were applied to 2 ml of herbal extract. Carbohydrates are present with purple and reddish colour. Add 1ml of Fehlings A and 1 ml of Fehlings B and boil for 10 mins. The reddish-brown color indicates the presence of carbohydrates.

b. Test for Tannins 2 ml of 5% ferric chloride was added to 1ml of herbal extract. The formation of dark blue or greenish-black indicates the presence of tannins.

c. Test for Saponins 2 ml of distilled water was added to 2 ml of plant extract and shaken 15 minutes longitudinally in a graduated cylinder. A foam layer of 1 cm indicating the presence of saponin is formed.

d. Test for flavonoids One ml of 2N sodium hydroxide was added to 2 ml of plant extract. The presence of flavonoids is indicated by the yellow colour.

e. Test for alkaloids Add 2 ml of concentrated hydrochloric acid to 2 ml of plant extract. Then there were added few drops of Mayer's reagent. The appearance of green colour or white precipitate indicates the presence of alkaloids.

f. Test for quinones Add 1 ml of concentrated sulphuric acid to 1 ml of plant extract. The red colour formation indicates that quinones are present.

g. Test for glycosides 3 ml of chloroform and 10% ammonia solution have been added to 2 ml of plant extract. The pink colour formation suggests that glycosides are present.

h. Test for cardiac glycosides To 0.5 ml of extract, 2ml of glacial acetic acid and few drops of 5% ferric chloride was added. This was under layered with 1 ml of concentrated sulphuric acid. The brown ring formation at the interface suggests the presence of cardiac glycosides.

i. Test for terpenoids Add 2 ml of chloroform to 0.5 ml of extract, and carefully add concentrated sulphuric acid. The red-brown colour formation at the interface indicates the presence of terpenoids.

j. Test for triterpenoids 1 ml of Libemann–Buchard Reagent (acetic anhydride + concentrated sulphuric acid) has been added to 1.5 ml of extract. The blue-green colour formation suggests that triterpenoids are present.

k. Test for phenols To A few drops of Phenol-Ciocalteau's reagent have been applied to 1 ml of plant extract, accompanied by a few drops of 15% sodium carbonate solution. The blue or green colour formation reveals the presence of phenols.

l. Test for coumarins Add 1 ml of 10% NaOH to 1 ml of plant extract. The yellow colour formation suggests that coumarins are present.

m. Steroids and phytosteroids To 1ml of plant extract, equivalent chloroform volume is added and subjected with just a few drops of concentrated sulphuric acid and appearance of the brown ring suggests that steroids are present and appearance of the bluish brown ring suggests the presence of phytosteroids.

n. Phlobatannins Few drops of 2% HCL were added to 1ml of plant extract, which indicate the presence of phlobatannins as an appearance of a red colour-precipitation.

o. Anthraquinones A few drops of 10% ammonia solution have been applied to 1 ml of plant extract, pink colour precipitation indicates that anthraquinones are present.

PHYTOCHEMICAL PROFILING

Estimation of Total Flavonoids

The total flavonoid content in the plant extracts was calculated using slight modification in the aluminium chloride spectrophotometric assay described by *Sankanaka et al.*,2005 [8]. The test is based on the quantification of the yellow orange colour, which results from the flavonoids and $AlCl_3$ reactions. In 10 mg / mL of the solvent dissolution a stock of plant extracts and regular (quercetin) was prepared. Quercetin grade (0.2–1.0 mg / mL) concentrations were prepared and 0.1 mL (Quercetin) and plant extracts were pipetted out into separate test tubes. Then, 0.9 mL of distilled water and 75 µL of 5% NaNO2 were added to the test tubes. This mixture was vortexed and given 6 minutes to stand. After 6 minutes, the mixture was added with 150 µL of 10% $AlCl_3$ and 0.5mL of 1M NaOH was added 5 minutes later. The solution was made up to 2.5 mL with the addition of distilled water and

20 minutes incubated at 40 °C. After incubation, spectrophotometer was used to record the absorbance of the pipetted mixture at 510 nm. Total flavonoid content was estimated as V. absorbance was immediately measured at 510 nm using a spectrophotometer µg/mg of quercetin equivalent (QE/mg) from the standard calibration curve y=0.0078x+0.1528, R^2=0.9968 using the equation [CV/M]. C is the extrapolated concentration from the standard linear graph, V is the volume of extract in mL and M is the mass of the extract used expressed in gram (mg).

Estimation of Total Phenolic Content

The total phenolic content of the extract was determined with some modifications by Folin-Ciocalteu's method. Gallic acid prepared as 1mg/mL was the standard. About 10 mg of the crude extract was dissolved in their respective solvents and the standard's concentration was graded in a series of 0.2–1.0mg/mL. 5 mL of the Folin-Ciocalteu reagent was added to 1 mL of each plant extract and standards in separate test tubes. Then, 4mL of anhydrous Na_2CO_3 (7.5% w/v) was added. The whole mixture was vortexed and incubated for 30 minutes at 40 °C. The absorbance was measured in a UV-Visible Spectrophotometer at 765 nm immediately after incubation. The total phenolic content was calculated as µg of Gallic acid equivalent (GAE) for each milligram of crude extract by the standard linear graph y=0.0107x−0.0221, R^2=0.9577 using the above-mentioned equation (CV/M).

Estimation of Total Tannin Content

The total tannin content was evaluated by the Folin - Ciocalteu method with minor modifications. 0.1mL each of the stock (extracts and standard) was added to the test tube containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent, 1 ml of 35 % Na_2CO_3. It was made up to 10 ml with distilled water. The resulting solution was vortexed and incubated at room temperature for 30 min. A set of reference standard solutions of tannic acid (20, 40, 60, 80 and 100 µg/ml) was also used. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The total tannin content was measured in terms of mg of Tannic acid equivalence (TE) µg/mg of the crude extract obtained from the y=0.004x+0.0052 calibration curve, R^2=0.9818 of the above-mentioned formula.

IV. RESULTS AND DISCUSSION

Qualitative Phytochemical Analysis

The medicinal value of plants lies in certain chemical substances which have a particular physiological effect on the human body. Various phytochemicals have been described as having a broad range of activities that can help to protect against persistent diseases [9].

In the present study, the phytochemical analysis was carried out in the leaf extracts of *W. somnifera* with different solvents such as hexane, ethyl acetate and ethanol. All results of the phytochemical analysis are showed in Table 1. The results confirmed that the phytochemical constituents such as tannins, saponins, flavonoids, and phenols present in all three extracts.

Estimation of Total Flavonoid Content

Flavonoids are present in the form of polyphenolic compounds with strong antimicrobial activity, anti-inflammatory activity. They avoid oxidative cell damage and have a significant anticancer activity [10].

In this study, this was expressed as **µ**g QE/mg of tested samples as shown in Fig. 1 below. The hexane extracts yielded more flavonoids than ethyl acetate and ethanolic extracts. The flavonoid values ranged from 9.92±0.68 in hexane followed by 8.87±1 in ethyl acetate and 3.65±0.7 in ethanol (**µ**g QE/mg)

Estimation of Total Phenolic Content

Terpenoids are a variety of compounds used by humans in food and pharmaceuticals. Phenols are the main group of plant metabolites with many biological properties, such as anti-apoptosis, anti-aging, anti-carcinogens, anti-inflammation and cell proliferation [11].

The total phenolic content was expressed as **µ**g/mg of the test samples in Gallic acid equivalent (GAE) as presented in Fig. 2 below. In all extracts, phenolic contents were higher in ethyl acetate extract with 0.82±0.2 followed by hexane with 0.65±0.03 and then ethanolic extract with 0.4±0.08.

Estimation of Total Tannin Content

Tannins have astringent properties that promote the healing of wounds and inflamed mucous membrane due to their physiological activities such as antioxidant, antimicrobial and anti-inflammatory properties [12]. *W. somnifera* contains numerous alkaloids to treat anti-inflammatory, immuno-modulatory and cytotoxic activity [13]. Alkaloids were absent in the *W. somnifera* methanolic extract but it also showed good antibacterial activity due to its flavonoid and tannin contents. Phytoconstituents have been screened for the methanol crude extract of *Withania somnifera* leaves (Solanaceae) which reveal the presence of flavonoids, steroids, alkaloids, saponins and tannins [14].

In our study, the total tannin content was expressed as µg/mg of the test samples in Gallic acid equivalent (GAE) as presented in Fig. 3 below. In all extracts, tannin contents were higher in an ethanolic extract with 13.9±1.3 followed by ethyl acetate with 13.2±1.5 and then hexane extract with 7.95±0.5.

V. CONCLUSION AND FUTURE SCOPE

Chemical content and bioactive fractions of medicinal plants are important and are considered to treat various diseases from olden days. *W. somnifera* participate as an important plant and is prescribed for treating many ailments that affect humans. Different parts of the plant were used to treat various diseases like anti-cancer, neuroprotective, anti-depressant, antioxidant, anti-inflammatory and anxiolytic, etc. Results from this study

suggest that the presence of major phytochemicals of *W. somnifera*. The biological active phytochemicals were present mostly in the Ethyl acetate extract of *W. somnifera*. However, there is a need for further research to explore *W. somnifera* towards drug development to its available commercial formulations.

Conflict of Interest

The authors declare that they have no conflict of interest in the publication.

Figures and Tables

Table 1. Phytochemical Screening of *W. somnifera* leaf extracts.

S. No	Tests	Hexane	Ethyl acetate	Ethanol
1	Carbohydrates	-	+	-
2	Tannins	+	+	+
3	Saponins	+	+	+
4	Flavonoids	+	+	+
5	Alkaloids	+	+	-
6	Quinones	-	-	+
7	Glycosides	-	-	-
8	Cardiac glycosides	-	-	+
9	Terpenoids	-	+	-
10	Phenols	+	+	+
11	Coumarins	+	+	-
12	Phytosteroids	+	-	-
13	Phlobatannins	-	-	-
14	Anthraquinones	-	-	-

(+) = Positive (-) = Negative

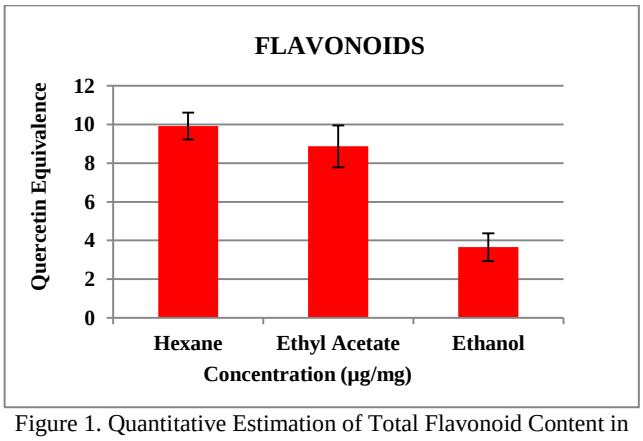

Figure 1. Quantitative Estimation of Total Flavonoid Content in *W. somnifera* Leaf Extract

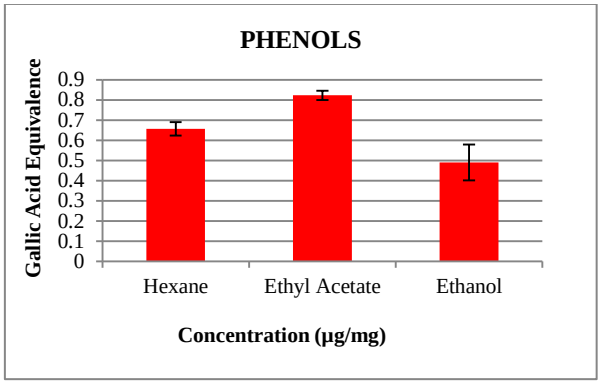

Figure 2. Quantitative Estimation of Total Phenolic Content in *W. somnifera* Leaf Extract

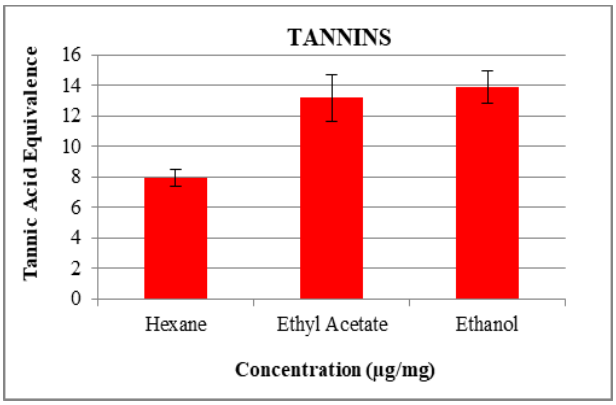

Figure 3. Quantitative Estimation of Total Tannin Content in *W. somnifera* Leaf Extract

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Sridhar Ramachandran had qualified UGC-NET lectureship in education (2011), a University rank holder, has had higher education from Chennai, Tamil Nadu Teacher Education and PRIST Universities. He contributed more than 10 research articles to National and International Journal. He had Gene Accession Numbers (GU479040 and J X 444058) from GenBank, National Centre for Biotechnology information (NCBI), USA. He was a chapter author in ISBN edited Research book-Contemporary Researches in Education (CRP), NAS publisher, Kerala India. He is an active life member in International Association of Medical and Pharmaceutical Virologists. Mr. Sridhar Ramachandran is associated with research work in Review of Progress - International Research Journal (ISSN No: 2321-7871) as An Advisory Board Member. He is currently pursuing his doctoral degree at Tamil Nadu Open University, Chennai.

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