Biolife 2(1):306-312
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
eISSN (online): 2320-4257
www.biolifejournals.com/home.html
BIOLIFE ORIGINAL ARTICLE

PRELIMINARY PHYTOCHEMICAL STUDIES OF MEDICINAL PLANT DRUG: *WITHANIA SOMNIFERA* LINN

Saidulu Ch[1*], Venkateshwar C[2] and Gangadhar Rao S[3]

[1,2,3]Department of Botany, University College of Science, Osmania University, Hyderabad-500007, Andhra Pradesh, India.

E-mail: saidulu.chilumula@gmail.com

ABSTRACT

Withania somnifera Linn is the most important plant commonly known as Ashwagandha belongs to the family solanaceae. It is an important medicinal plant that has been used in Ayurvedic and indigenous medicine for over 3,000 years. Ashwagandha contains alkaloids as well as steroidal lactones. Anahygrine, Anaferine, sominiferine, sominiferinine, withanine and withananine are chemical compounds present in ashwagandha. Ashwagandha is used in asthma, bronchitis, rheumatoid arthritis. Secondary metabolites present in medicinal plants are responsible for curing various diseases, Phytochemical screening revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, coumarins, quinines, cardiac glycosides, xantho proteins, glycosides, steroids, phenols, resins, carboxylic acid groups in varying concentrations and the maximum solubility of all the phytochemicals was observed in methanol, water and chloroform extractions, but resins, coumarins are absent in the petroleum ether, acetone, and also coumarins, carboxylic acid, quinines, xantho proteins are completely absent in the Petroleum ether. The aim of the present study was to evaluate the qualitative analysis of *Withania somnifera* phytochemicals in various solvents such as methanol, chloroform, petroleum ether, acetone and distilled water

Key words: *Withania somnifera*, Phytochemical constituents, Crude extract, Solvents.

INTRODUCTION

Withania somnifera (Linn), is an erect, evergreen, perennial shrub which is a widely used medicinal plant considered as aphrodisiac and rejuvenating, anti-inflammatory and anti tumouragent (Naidu PS, *et al.*, 2003). It is widely used as an important drug in Ayurvedic formulations. The genus *Withania somnifera* belongs to the division Magnoliophyta, class magnoliopsida, order solanales and family Solanaceae. (Heiser, Smith., 1953). It is best known classically for its rejuvenating properties, and hence called "Indian Ginseng" (Singh and Kumar, 1998). Roots of *Withania somnifera* (Ashwagandha) reportedly exhibit antioxidant, immune modulatory and haematopoietic properties (Mishra LC, *et al.*, 2000). Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants,which provide health benefits for humans than those attributed to macronutrients and micronutrients (Hasler *et al*,1999). In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack a recalled as phyto chemical (Gibson EL, *et al*., 1998; Mathai K, *et al.*, 2000). They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. Phytochemical accumulate in different parts of the plants, such as in the roots, stems and leaves (Costa MA, Zia ZQ, *et al.*, 1999; Rajeshwar and

Lalitha, 2013). These compounds are known as secondaryplant metabolites and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anti cancer property.

The active pharmacological components of *Withania somnifera* constituents are withanolides (Steroidal lactones with ergostane skeleton) and alkaloids (Elsakka M, *et al.,* 1990). It also possesses antistress, immune modulatory, anti-oxidant and antibacterial activity (Kupchan SM, *et al.,* 1965; Devi PU, Sharada AC, *et al.,* 1992; Devi PU, *et al.,* 1993 and Lingaiah and Nagaraja Rao, 2013).The plant has been found useful in the treatment of burns, wounds, and dermatological disorders, and gastrointestinal diseases, dysfunctions of the respiratory system, asthma, bronchitis, cancer and geriatric problems (Grierson, D.S, *et al.,* 1999). Phytochemical screening of the extracts was also carried out to assess the presence of different phytochemical in different extracts (Devmurari V. P *et al.,* 2010).

MATERIAL AND METHODS

Plant material source:
Withania somnifera seeds were procured from the CIMAP, Hyderabad and sown the seeds in earthen pots at Green house of Botanical Garden, Department of Botany, Osmania University, Hyderabad, A.P, India.

Solvents for Extraction:
1) Methanol 2) Petroleum ether 3) Acetone 4) Distilled Water and 5) Chloroform.

Preparation of crude extracts:
The air dried root, leaf and stem were milled to get a coarse powder. About 100g of dry powder was extracted with petroleum ether at room temperature using soxhlet apparatus for 8hrs.or the extraction was continued until the liquid was clear. The extracts were then filtered and concentrated to a dry mass under vacuum .The marc left after petroleum ether extraction was air dried and then extracted with different solvents chloroform, Acetone, Distilled Water and methanol as done earlier and the extracts were similarly filtered and concentrated under vacuum (waltor, 1972).

Qualitative chemical evaluation:
The different extracts thus obtained were qualitatively tested for the presence of various phytochemical constituents (Brain and Turner, 1975; Sofowora, 1982; Treas and Evans, 1983, Kepm, 1986; Harbone, 1991).

1. Detection of Flavonoids (Ferric chloride test)
Ferric chloride test: A few drops of neutral ferric chloride solution were added to one ml each of above alcoholic solution. Formation of blackish red colour indicates the presence of flavonoids.

2. Detection of Alkaloids (Wagner's test)
Wagner's test: to the acidic solution, Wagner's reagent (iodine in potassium iodide) was added. Brown precipitate indicates the presence of alkaloids. (I_2=1.27gm, KI=2gm+5ml H_2O final makeup 100ml)

3. Detection of Glycosides
A small amount of alcoholic extract of samples was dissolved in 1ml water and then aqueous 10% sodium hydroxide was added. Formation of a yellow colour indicated the presence of glycosides.

4. Detection of Steroids (Salkowski's test)
About 100 mg of dried extract was dissolved in 2m l of chloroform**.**Sulphuric acid was carefully added to form a lower layer. A reddish brown colour at the interface was an indicative of the presence of steroidal ring.

5. Detection of Phenols (Ferric chloride test)
One ml each of the various extracts dissolved in alcohol or water was separately treated with a few ml of neutral ferric chloride solution. Any change in colour indicates the presence of phenols.

6. Detection of Terpenoid (Salkowski's test)
2ml of chloroform and 1ml of conc.H_2SO_4 was added to 1mg of extract and observed for reddish

brown colour that indicates the presence of terpenoid.

7. Detection of Saponins

A drop of sodium bicarbonate was added in a test tube containing about 50ml of an aqueous extract of samples. The mixture was shaken vigorously and kept for 3min. a honey comb like froth was formed and it shows the presence of saponins.

8. Detection of Resins

One ml of various extracts was diluted with water. Formation of bulk black precipitate indicates the presence of resins.

9. Detection of Tannins (Ferric chloride test)

Ferric chloride test: to the filtrate, a few drops of ferric chloride solution were added. A blackish precipitate indicates the presence of tannins

10. Cardiac Glycosides (Keller Killiani's test)

About 100mg of extract was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution. This was then underlayer with 1ml of conc. Sulphuric acid. A brown ring obtained at the interface indicated the presence of a deoxy sugar characteristic of cardenolides.

11. Detection of Carboxylic acid

One ml of the various extracts was separately treated with a few ml of sodium bicarbonate solution. Effervescence (due to liberation of carbon dioxide) indicates the presence of carboxylic acid.

12. Detection of Coumarins

On ml of each various extracts was treated with alcoholic10% sodium hydroxide. Dark yellow colour shows the presence of coumarins.

13. Detection of Quinones

One ml of each of the various extracts was treated separately with alcoholic potassium hydroxide solution. Quinines give coloration ranging from red to blue.

14. Detection of Xantho proteins

One ml each of the various extracts was treated separately with few drops of conc. HNO_3 and NH_3 solution. Formation of reddish orange precipitate indicates the presence of xantho proteins.

RESULTS AND DISCUSSION

Preliminary Phytochemical screening of *Withania somnifera* root:

Preliminary Phytochemical screening of the methanol, chloroform and distilled water extracts of root powder of *Withania somnifera* revealed the presence of flavonoids, alkaloids, glycosides, steroids, phenols, terpenoids, saponins, resins, tannins, cardiac glycosides, carboxylic acids, coumarins, quinones, xantho proteins. Acetone extract of root powder of *Withania somnifera* revealed the presence of flavonoids, alkaloids, glycosides, steroids, phenols, terpenoids, saponins, tannins, cardiac glycosides, carboxylic acids, coumarins, quinones, xantho proteins. Petroleum ether extracts also possess those phytoconstituents except Carboxylic Acids, Coumarins, Quinones, Xantho Proteins. (Table No: 1)

Preliminary Phytochemical screening of *Withania somnifera* leaf:

Methanol extracts, chloroform extracts and distilled water extracts of leaf powder of *Withania somnifera* contains flavonoids, alkaloids, glycosides, steroids, phenols, terpenoids, saponins, resins, tannins, cardiac glycosides, carboxylic acids, coumarins, quinones, xantho proteins. Acetone extract contains flavonoids, alkaloids, glycosides, steroids, phenols, terpenoids, saponins, tannins, cardiac glycosides, carboxylic acids, coumarins, quinones, xantho proteins. Petroleum ether extracts also possess those phytoconstituents except carboxylic acids, xantho proteins. (Table No: 2)

Preliminary Phytochemical screening of of *Withania somnifera* stem:

Preliminary Phytochemical screening of the methanol, chloroform extracts of stem powder of *Withania somnifera* showed the presence of flavonoids, alkaloids, glycosides, steroids,

Table-1: Preliminary phytochemical analysis of various extracts of *Withania somnifera* root

S.N	Phytochemical Constituents	Petroleum Ether	Chloroform	Acetone	Methanol	Distilled Water
1.	Flavonoids	+	+	++	++	++
2.	Alkaloids	++	+++	+++	+++	+++
3.	Glycosides	+	+	+	++	++
4.	Steroids	++	++	+++	+++	++
5.	Phenols	+	+	++	++	++
6.	Terpenoids	++	++	+++	+++	++
7.	Saponins	+	+	++	++	+
8.	Resins	-	+	-	+	+
9.	Tannins	+	+	++	++	++
10.	Cardiac Glycosides	++	+	+++	+++	+++
11.	Carboxylic Acids	-	+	+	++	++
12.	Coumarins	-	+	-	++	++
13.	Quinones	-	+	+	++	++
14.	Xantho Proteins.	-	+	++	+++	++

(-)=absent, (+) =present, (++) =moderately present, (+++) =Appreciable amount

Table-2: Preliminary phytochemical analysis of various extracts of *Withania somnifera* leaf

S.N	Phytochemical Constituents	Petroleum Ether	Chloroform	Acetone	Methanol	Distilled Water
1.	Flavonoids	+	++	+	++	++
2.	Alkaloids	+++	+++	+++	++	+++
3.	Glycosides	+	++	+	++	++
4.	Steroids	++	+++	+++	++	+
5.	Phenols	+	++	+	+	++
6	Terpenoids	+	+++	+	+	++
7.	Saponins	+	++	+	+	++
8.	Resins	-	+	-	+	+
9.	Tannins	+	+	+	++	++
10.	Cardiac Glycosides	++	++	+++	+++	++
11.	Carboxylic Acids	-	+	+	+	++
12.	Coumarins	-	++	-	+	++
13.	Quinones	-	+	+	++	++
14.	Xantho Proteins	-	++	+	++	++

(-)=absent, (+) =present, (++) =moderately present, (+++) =Appreciable amount

Table-3: Preliminary phytochemical analysis of various extracts of *Withania somnifera* stem

S.N	Phytochemical Constituents	Petroleum Ether	Chloroform	Acetone	Methanol	Distilled Water
1.	Flavonoids	+	+	+	+++	++
2.	Alkaloids	++	+++	+++	+++	+++
3.	Glycosides	+	+	+	++	++
4.	Steroids	++	++	++	+	+
5.	Phenols	+	+	+	++	+
6.	Terpenoids	++	++	++	+	+
7.	Saponins	+	+	+	+	+
8.	Resins	-	+	-	+	-
9.	Tannins	+	+	++	+++	+++
10.	Cardiac Glycosides	++	++	+	+	+
11.	Carboxylic Acids	-	+	++	++	++
12.	Coumarins	-	+	-	+	+
13.	Quinones	-	+	+	+	+
14.	Xantho Proteins	-	+	++	++	++

(-)=absent, (+) =present, (++) =moderately present, (+++) =Appreciable amount

Figure-1. Phytochemical screening of root extracts of *Withania somnifera* in various solvents

Figure-2. Phytochemical screening of leaf extracts of *Withania somnifera* in various solvents

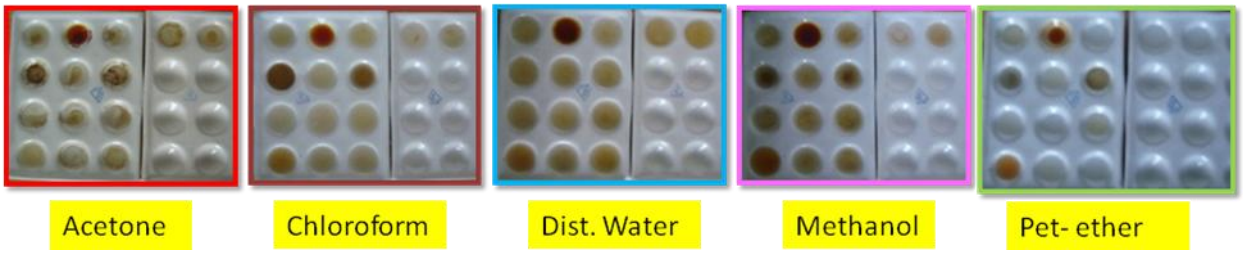
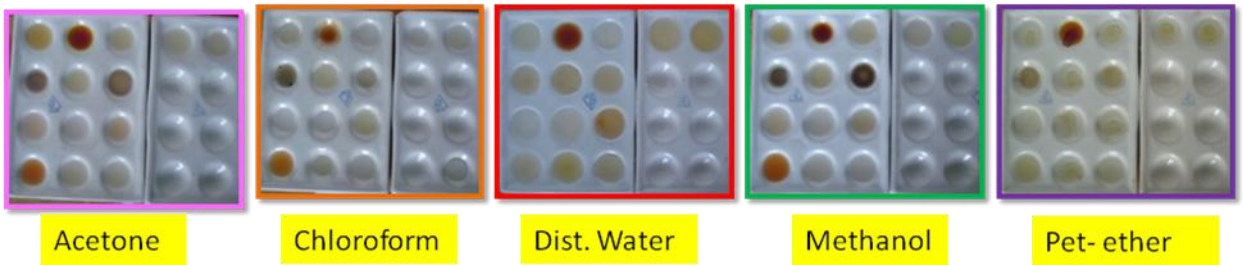


Figure-3. Phytochemical screening of stem extracts of *Withania somnifera* in various solvents



phenols, terpenoids, saponins, resins, tannins, cardiac glycosides, carboxylic acids, coumarins, quinones, xantho proteins. Distilled Water extract of stem powder of *Withania somnifera* showed the presence of flavonoids, alkaloids, glycosides, steroids, phenols, terpenoids, spooning, tannins, cardiac glycosides, carboxylic acids, coumarins, quinines, xanthus proteins. Acetone extracts also possess those phytoconstituents except coumarins. Pet-ether extract contains flavonoids, alkaloids, glycosides, steroids, phenols, terpinoids, saponins, tannins, cardiac glycosides. (Table No: 3).

CONCLUSION

The present study may be useful to supplement the information with regard to its standardization and identification and in carrying out further research and its use in Ayurvedic system of medicine. The use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases. Based on the results of this study it may be concluded that Methanol, Chloroform and Distilled Water extracts of *Withania somnifera* revealed the maximum presence of alkaloids, flavonoids, terpenoids, tannins, glycosides, steroids, phenols, saponins, resins, quinines, coumarins, cardiac glycosides, carboxylic acid, xantho proteins.

ACKNOWLEDGMENT

This research was supported by DST-PURSE, Research Project Ltr No. A-46/BOT//DST-PURSE/2011, New Delhi, India is greatly acknowledged. I am very thankful to Prof. B. Sashidhar Rao Coordinator, OU-DST PURSE Programme, Department of Biochemistry, Osmania University and Hyderabad for his support.

REFERENCES

1. **Costa MA, Zia ZQ, Davin LB, Lewis NG**. 1999. Chapter Four: Toward Engineering the Metabolic Pathways of Cancer-Preventing Lignans in Cereal Grains and Other Crops. In Recent Advances in Phytochemistry, vol. 33, Phytochemicals in Human Health Protection, Nutrition, and Plant Defense, ed. JT Romeo, New York, 67-87.
2. **Devmurari V. P. and Jivani N. P**, 2010.Annals *of Biological Research*, 1 (1), 10-14.
3. E**lsakka M, Grigorescu E, Stanescu U, Stanescu U, Dorneanu V.** 1990. New data referring to chemistry of *Withania somnifera* species. Rev Med ChirSoc Med Nat Iasi.; 94:385–387.
4. **Grierson, D.S. and Afolayan, A.J**.1999**.** *J.Ethnopharmacol*, , 66, 103-106.
5. **Gibson EL, Wardel J, Watts CJ.** 1998. Fruit and Vegetable Consumption, Nutritional Knowledge and Beliefs in Mothers and Children. Appetite; 31: 205-228.
6. **Heiser, C.B and Smith P.C.,** 1953.The cultivated Capsicum. Econ. Bot.1: pp: 214-227.
7. **Harborne JB.** 1984. Phytochemical Methods: A Guide to ModernTechniques of Plant Analysis, 2nd.
8. **Harborne JB, Baxter H**. 1995. Phytochemical dictionary: a handbook of bioactive compounds from plants. 4 John St. London: Taylor & Francis Ltd.
9. **Harborne JB, 1984."**Phytochemical methods" (2nd Ed). Chapman and Hall, London and New York .pp288.
10. **Hasler CM, Blumberg JB**. 1999. Symposium on Phytochemicals: Biochemistry and Physiology. Journal of Nutrition; 129: 756S-757S.
11. **Kupchan SM, Doskotch RW, Bollinger P, Muphail AT, Sim GA and Saenz RJ**. 1965. The isolation and structure elucidation of a novel steroidal tumor inhibitor from *Acnistus arborescens*. J. Am. Chem. Soc; 87:5805.
12. **Lingaiah M and Nagaraja Rao P**. 2013. An ethnobotanical survey of medicinal plants used by traditional healers of Adilabad district, Andhra Pradesh, India. Biolife.1 (1):17-23.
13. **Mathai K**. 2000.Nutrition in the Adult Years. In Krause's Food, Nutrition, and Diet Therapy, 10th ed., ed. L.K. Mahan and S. Escott-Stump, American Cancer Society.

: 271: 274-275.
14. **Mishra LC, Singh BB and Dagenais S**. 2000. Scientificbasis for the therapeutic use o*f Withania somnifera* (Ashwagandha), a review. Altern. Med. Rev.; 5:334-346
15. **Naidu PS, Singh A and Kulkarni SK**, 2003. Effect of *Withania somnifera* root extract on haloperidol induced orofacial dyskinesia: Possible mechanism of action. J. Med. Food,; 6: 107-114.
16. **Rajeshwar Y and Lalitha R.** 2013. Preliminary phytochemical screening and in vitro anthelmintic effects of *Acmella paniculata* plant extracts. Biolife. 1(3):-106-112.
17. **Singh, S., Kumar S.,** 1998. *Withania somnifera* – The Indian Ginseng Ashwagandha. Central Institute of Medicinal and Aromatic Plants.
18. **Sharma S, Sharma MC and Kohli DV**. 2009. *In vitro* micro propagation of medicinally important roots and axillary bud combination. Journal of Optoelectronic and biomedical materials; 4: 379-381.
19. **Sofowora A**. 1993.Medicinal Plants and Traditional Medicine in Africa. 2nd ed., Spectrum Books Ltd., Ibadan, Nigeria.
20. **Treas,G.E.andEvans,**1985."Pharmacognoc y", English language Book Society,Bailliere Tindall,London.pp:394.

DOI:https://dx.doi.org/10.5281/zenodo.7197251
Received: 11 January 2014;
Accepted; 23 February 2014;
Available online : 5 March 2014