ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info/jasr

ISSN
0976-9595
Short Communication

EVALUATION OF PHYTOCONSTITUENTS OF METHANOLIC ROOT EXTRACT *OF WITHANIA SOMNIFERA*

Shenkreppa Doddappa Desai[1], Gavishiddappa Andanappa Hadimani[2]*, Ishwar Basvantappa Bagoji[2], Prakash Biradar[3], Nanjappaiah H M[3], Shivakumar Hugar[3]

[1]*Shridevi Institute of Medical Science & Research Hospital, Tumkur*

[2]*Dept. Of Anatomy, Shri B M Patil Medical College, Hospital and Research Centre BLDE University, Bijapur*

[3] *Dept. of Pharmacology BLDEA's College of Pharmacy, Bijapur*

Corresponding author: gavish.hadimani@yahoo.com, gavish.hadimani@bldeuniversity.ac.in

ABSTRACT

The present research work was designed to identify the phytochemical constituents of methanolic extract of roots of *Withania somnifera*. *Withania somnifera* is widely used in Ayurvedic medicine, the traditional medical system of India. It is a member of the family Solanaceae. Therapeutic importance of the different parts of this plant has a long history. Fresh mature roots were shade dried at room temperature, coarse powdered and extracted with methanol by Soxhlet's extraction method. Thereafter, the extract was concentrated using rotary flash evaporator to obtain semisolid crude extract with the yield of 10.682%. *Withania somnifera* root extract was investigated for the presence of phytochemical constituents. The preliminary phytochemical evaluation of the *Withania somnifera* root extract revealed the presence of alkaloids, flavonoids, phenolics, carbohydrates, tannins, steroids and starch.

Keywords: *Withania somnifera*, Methanolic extraction, Phytochemicals

1. INTRODUCTION

Nature always stands as a golden mark to exemplify the outstanding phenomenon of symbiosis. The biotic and abiotic elements of nature are all interdependent. The quest for long, healthy and happy life is as old as man himself. Nature has provided a complete storehouse of remedies to relieve the ailments of mankind. The consistent effects have resulted in many effective means of ensuring health care. The seers of Ayurveda were able to understand and record the various aspects regarding the drugs that even today are difficult to understand with modern available parameters [1, 2].

Withania somnifera (*W. somnifera*, Ashwagandha) is widely used in Ayurvedic medicine, the traditional medical system of India. Its height is 3-4 feet and grows into a bush and is a member of the family Solanaceae. In India, its growth is maximum and at present this plant is cultivated for medicinal purpose. Therapeutic importance of the different parts of this plant has a long history and is mentioned in Charak Sanghita. It is an ingredient in many formulations prescribed for a variety of musculoskeletal condition (e.g., arthritis, rheumatism) and as a general tonic to increase energy, improve overall health and longevity and prevent disease in athletes, the elderly and during pregnancy. It is also used as an anti stressor and antioxidant agent [3, 4]. Number of reactive molecules generated through various biological redox reaction such as superoxide radicals (O_2-), hydroxyl radicals (OH), hydrogen peroxide (H_2O_2) and nitric oxide $(NO)^2$ which can directly react with biological macromolecule such as proteins lipid and DNA of health human cells and cause cell membrane disintegration, DNA mutation and protein damage deregulation of these reactive oxygen species (ROS) can further create cancer, atherosclerosis, cardiovascular disease, liver injury, ageing and inflammatory disease [5]. Antioxidants act as oxygen scavengers by interrupting the oxidation process by reacting with free radical, chelating catalytic metals [6]. Some synthetic antioxidants were developed in the past few decades but they are suspected of having some adverse effects. Therefore in search of suitable alternative natural antioxidants has received much attention to identify and develop more potent antioxidants of natural origin to replace synthetic ones. Different kinds of plant material have already been reported as natural antioxidants [7].

Ashwagandha root was used historically as an aphrodisiac. This herb is mentioned in the ancient Kama Sutra as an herb to be used for heightening sexual experience, this herb has the ability to restore sexual health and improve overall vitality while promoting a calm state of mind. Laboratory studies show it can produce nitric oxide which is known to dilate blood vessels. Hence, in the present study, the roots of *Withania somnifera* were investigated and their chemical composition was investigated with a view to assess their phytochemical potential.

2. MATERIAL AND METHOD

2.1. Drug preparations

2.1.1. Preparation of plant material

The roots of *Withania somnifera* were procured locally; plant was authenticated by Dr. M B Mulimani Professor, Department of Botany. SB Arts & KCP Science College, Bijapur by the studies include organoleptic tests and macroscopic and microscopic observations. After authentication the voucher specimen was deposited in the Department.

2.1.2. Methanolic extraction of Withania somnifera

Roots of *Withania somnifera* were washed twice using tap water and then washed again in distilled water to remove the dust. The roots were shade dried for 7–12 days at room temperature, until they were free from the moisture and then pulverized into coarse powder. The powdered root extracted with methanol by Soxhlet's extraction method. Thereafter, the extract was concentrated using rotary flash evaporator to obtain semisolid crude extract. The percentage yield of the extract was found to be 10.682%. The extract was stored in airtight container in refrigerator below 10^0C. Desired concentration of stock solution was prepared using distilled water for preliminary phytochemical investigation.

2.2. Preliminary phytochemical screening

A systematic and complete study of crude drugs includes a complete investigation of both primary and secondary metabolites derived from plant metabolism. Different qualitative test were performed for establishing profiles of various extracts for their nature of chemical composition. The extracts obtained were subjected to following chemical tests for identification of various phytoconstituents as per the methods given by Harborne [9]. There were no previously isolated compounds.

2.2.1. Test for starch

Dissolved 0.015 gm of iodine and 0.075 gm of potassium iodide in 5 ml of distilled water and add 2-3 ml of an aqueous extract of drug, blue color is produced.

2.2.2. Test for Steroids [10]

Salkowski test: 2-3 drops of concentrated sulphuric acid was added to chloroform solution, shaken and allowed to stand, appearance of red color in lower layer indicates the presence of sterols.

Liebermann-Burchard test: Extract was mixed with the chloroform and few drops of acetic anhydride and mixed well. Concentrated sulphuric acid was added from the sides of the test tube slowly until the ring appears; appearance of reddish brown ring indicates the presence of steroids.

2.2.3. Test for Flavonoids [11, 12]

Shinoda test: To the extract a few fragments of magnesium ribbon and concentrated hydrochloric acid was added. Appearance of red to pink color after few minutes indicates the presence of Flavonoids.

Lead acetate test: To the extract added few drops of aqueous basic lead acetate solution. Formation of yellow precipitate indicates presence of flavonoids**.**

Alkaline reagent test/ NaOH test: few drops of sodium hydroxide solution were added to extract. Intense yellow color disappeared after adding dilute HCl which indicates the presence of flavonoids.

2.2.4. Test for Alkaloids

The extract was basified with ammonia and extracted with chloroform. The chloroform solution was acidified with dilute hydrochloric acid, shaken well and filtered. The filtrate was used for testing the alkaloids.

Hager's test: The filtrate was treated with few drops of Hager's reagent. Formation of yellow precipitate indicates the presence of alkaloids [13].

Wagner's test: (Iodine in Potassium iodide): The acid layer was treated with few drops of Wagner's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

*Mayer's test***:** (Potassium Mercuric Iodine solution): The acid layer was treated with few drops of Mayer's reagent. Formation of creamy white precipitate indicates the presence of alkaloids.

*Dragendorff's reagent***:** (Potassium Bismuth Iodide): The acid layer was treated with few drops of Dragendorff's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

2.2.5. Test for Tannins [14]

Gelatin test: To the extracts of the drug added 1% solution of gelatin containing 10% sodium chloride. Formation of white precipitate indicates the presence of tannins.

Ferric chloride test: To extracts few drops of 1% neutral ferric chloride solution were added, formation of blackish blue color indicates the presence of tannins.

2.2.6. Test for Saponins [13, 15]

Foam test: Small amount of extract of the drug was shaken with little quantity of water, if foam produced persists for 10 minutes; it indicates the presence of saponins.

Froth test: To 5 ml of extract of the drug added single drop of sodium bicarbonate solution. Shaken the mixture vigorously and left for 3 minutes. Formation of honey comb like froth indicates presence of saponins.

2.2.7. Test for Carbohydrates:

Small amount of extracts of the drug were dissolved in little quantity of distilled water and filtered separately. The filtrates were used to test presence of carbohydrates.

Molisch's test: The filtrate of the drug was treated with Molisch reagent and concentrated sulphuric acid was added from the sides of the test tube to form a layer. A reddish violet ring shows the presence of carbohydrates.

Benedicts test: to the filtrate added 2 ml Benedict's reagent and boiled in water bath. Formation of Green or reddish brown precipitate indicates presence of carbohydrates.

Fehlings test: Filtrates were hydrolyzed with dilute hydrochloric acid, neutralized with alkali and heated with equal amount of

Fehling's A and B solutions. Formation of green to yellow to red precipitate indicated the presence of reducing sugars.

2.2.8. Test for Phenols

Phenolic compounds: Extract was dissolved in alcohol and 1 drop of neutral ferric chloride was added to this. The intense color indicates the presence of phenolic compound.

2.2.9. Glycosides

0.5ml of extract was taken in a test tube and added with 1 ml glacial acetic acid containing traces of ferric chloride. To this solution 1 ml of concentrated sulphuric acid was added and observed for the formation of reddish brown color at the junction of two layers and the upper layer turned bluish green in the presence of glycosides.

3. RESULTS

3.1. Preliminary phytochemical screening

Preliminary Phytochemical screening of methanolic extract of roots of *Withania somnifera* revealed the presence of different kind of phytochemical components that are summarized in table 1.

Table 1: Preliminary phytochemical screening of Methanolic root extract of *Withania somnifera*

S. N.	Phytochemical	Test	Result
1	Test for starch	Iodine test	Present
2	Test for Steroids	Salkowski test	Present
		Liebermann-Burchard test	Present
3	Test for Flavonoids	Shinoda test	Present
		Lead acetate test	Present
		Alkaline reagent test/ NaOH test	Present
4	Test for Alkaloids	Hager's test	Present
		Wagner's test	Present
		Mayer's test	Present
		Dragendorff's reagent	Present
5	Test for Tannins	Gelatin test	Present
		Ferric chloride test	Present
6	Test for Saponins	Foam test	Absent
		Froth test	Absent
7	Test for Carbohydrates	Molisch's test	Present
		Benedicts test	Present
		Fehlings test	Present
8	Test for Phenolics	Lead acetate test	Present
9	Test for Glycosides	Foam test	Absent

4. DISCUSSION

The phytochemical screenings of chemical constituents related to biological activity of the plant root extract are alkaloids, flavonoids, phenolics, carbohydrates and tannins (Table 1). The quantitative phytochemical estimation indicate that *W. somnifera* root extract contains significant amount of phenolic, flavonoids, carbohydrate, tannin and alkaloid content which confirms its antioxidant property [16]. Phenolics content are very important plant constituents because they can act as reducing agents, hydrogen donors and metal chelator [17]. They also act as radical scavenger due to their hydroxyl groups. Flavonoids show their antioxidant action through scavenging or chelating process [18]. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity [19]. Also, the carbohydrates in food are of major interest in relation to chronic diseases. Different types of carbohydrates give rise to different glycaemic responses, and also able to stimulate lipogenesis [20, 21]. Moreover, in the medicinal effects described in the ayurvedic, siddha, folk and chinese traditional recipe tannins, phenolic acids, flavonoids and alkaloids are the important ingredients to prevent against oxidative stress and decrease the activity of cholinesterase and xanthine oxidase and also alleviating the mucus secretion in the airway glands [22]. The results acquired in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and this plant is proving to be a valuable reservoir of bioactive compounds of substantial medicinal merit.

5. CONCLUSION

In conclusion, the findings of the present study suggest that roots extract of the *Withania somnifera* possesses alkaloids, flavonoids, phenolics, carbohydrates, tannins, steroids and starch.

6. ACKNOWLEDGEMENTS

The authors are thankful to Principal of BLDEA's College of Pharmacy, Bijapur for providing facility to carry out extraction and phytochemistry also thankful to Dr. M B Mulimani Professor, Department of Botany, S B Arts & KCP Science College, Bijapur for authenticating the drug. Our special thanks to Prof. Dr. Kusal K Das for his constant support and guidance.

7. REFERENCES

1. Charaka S, Chakrapani DTCP, Varanasi; 1992.
2. Govind DSR. Bhaishajya R, Chowkambha Sanskrit Series office, Varanasi; 1961.
3. Bone K. Clinical applications of Ayurvedic and Chinese herbs. Monographs for the Western herbal practioner. Aust Phytotherapy Press; 1996; p. 137-41.
4. Dadkar VN, Ranadive NU, Dhar HL. *Ind J Clin Biochem,* 1987; **2:**101-108.
5. Aruoma OI, Halliwell B, Hoey BM, Butter J. Free *Radic Biol Med*, 1989; **6(6):**593-597.
6. Braca A, Sortino C, Politi M, Morelli I, Mendez J. *J Ethnopharmacol*, 2002; **79(3):**379-381.
7. Gulcin I, Buyukokuroglu ME, Oktay M, Kufrevioglu OI. *J Pineal Res*, 2002; **33(3):**167-171.

8. Packer L, Ong ASH. Biological oxidants and antioxidants: Molecular mechanisms and health effects. Champaign, USA: AOCS Press; 1997.
9. Harborne JB, Phytochemical methods: A guide to modern techniques of plant analysis, 3rd edition, Chapman and Hall, London; 2007: 135-203.
10. Gibbs RD. Chemotaxonomy of Flowering Plants, Vol.1, McGill Queen's University Press, Montreal and London; 1974.
11. Peach K, Tracey MV. Modern methods of plant analysis, Vol 3, Springer Verlag, Berlin; 1956.
12. Rizk AM. Fitoterapia; 1982:52(2): 35-42.
13. Trease GE, Evans WC. Pharmacognosy, 15th edition, WB Saunders Publishers, London; 2002.
14. Kokate CK. Practical Pharmacognosy. 4th edition, Vallabh Prakashan, New Delhi, India; 1994.
15. Sofowara A. Medicinal plants and traditional medicine in Africa. Chichester John Wiley & Sons, New York; 1993.
16. Dipankar C, Nikhil BG, Rhitajit S, Nripendranath M. *Asian J. Pharm Clin Res*, 2012; **5(4):**193-199.
17. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. *Free Rad Res*, 1995; **22(4):**375-383.
18. Kessler M, Ubeaud G, Jung L. *J Pharm Pharmacol*, 2003; **55(1):**131-142.
19. Nobori, T., Miurak, K., Wu, D.J., Takabayashik, LA, Carson DA. *Nature*, 1994; **46:**753-756.
20. Weisburger JH. *Nutrition*, 2001; **16:**767-773.
21. Parks EJ. *Br J Nutr,* 2002; **87 (Suppl.2):** S247-S253.
22. Franova S, Nosalova G, Mokry J, Mahmud THK, Ather A. Phytotherapy of cough. In Iwu M (ed.) Advances in phytomedicine, U.S.A. Elsevier; 2006. p. 111-131.