

ISOLATION, PHYTOCHEMICAL ANALYSIS AND BIOLOGICAL STUDIES OF “WITHANIA SOMNIFERA” FRUITS OF DISTRICT BANNU, KHYBER PAKHTUNKHWA, PAKISTAN

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ABSTRACT

The current research aim with the study of *Withania somnifera* a best natural source of potent. This biologically important medicinal plant contain various natural product in all parts i.e.s in areal parts, flowers, leaves, stem and underground roots. The research project conducted for determining and exploration of different natural products by qualitative phytochemical screening test from various factions. Current study support that the plant contain many natural product in different factions. The research confirmed important natural products are present in Hexane, Chloroform, ethyl acetate, butanol and water fraction and can be extracted easily by different methods.

KEYWORDS

Physiochemical analysis, *Withania somnifera*, fruit and Bannu

INTRODUCTION

The Medicinal plants are biosynthetic laboratories for chemical compounds, plants are used for medicinal purposes and maximum of the world’s Societies are still consuming medicinal plants as preparations for several disorders. The use of medicinal herbs particularly in traditional medicine is now well accepted and recognized as a concrete occupation [1]. Extraction of active compounds from therapeutic plants allows the demonstration of its biological activity. It also helps pharmacological

studies resulting in production of an additional effective medication with reduced side effects [2]. Furthermore; the vigorous constituents of herbal preparations have the benefits of being joined with a lot of many other compounds that seem to be inactive. However, these paired constituents provide the herb as a whole a security and effectiveness, considerable greater than that of its isolated and pure active constituents. At present in the developing countries, artificially synthetic preparations are not only expensive and insufficient for the curing of illnesses but are also often with contamination and side-effects [3]. Therefore, there is the necessity for investigation for plants of pharmaceutical importance. In the preceding few years, there is an exponential progress in the field of plant isolated medicine, and these medicines are attaining attraction both in developing and developed nations for the reason that of their natural source and a reduced amount of side effects. *Withania somnifera* (Ashwagandha) is a perennial herb, native to the Indian subcontinent, effectively introduced worldwide, now a days widely cultivated in several other countries including India [4]. *Withania somnifera* belongs to family Solanaceae, is an arboreal flowering shrub generally called as “Indian Winter cherry” Or “Indian Ginseng” [5].

Withania somnifera is known as Ashwaganda in Ayurveda, the traditional Indian way of medicine has been regarded such as a Rasayana drug. *Withania somnifera* play a vital role in herbs of Ayurveda utilized for era as a Rasayana due to its various medical welfare importance. *Withania somnifera* is used to encourage physical and psychological condition by increasing resistance contrary to illnesses and harsh ecological factors, also used for controlling the aging, fortifying the body form in weakened circumstances and used as a motivating stimulant for frame of mind stabilization [6]. The bioactive glycosides of *withania somnifera*, comprising of sitoindosides-VII to X, and Withaferin-A earlier also found for prevention of serious stress-induced anxiety and long-lasting stress-induced depression in rats [7,8]. Rasayana referred to a herbal assessment which stimulates new condition of physical and emotional condition that increase happiness. Ayurveda Rasayana herbs has the utmost noticeable occupancy [9]. Some Rasayana plants have adaptogen/anti-stress compounds. Ashwaganda is locally found churna, which in mixture with water, clarified butter or wax and is fine separated powder. Boosting of the memory expands the function of brain and nervous systems have done by it. *W. somnifera* fruits coagulate and increase the nutritional value of the milk when taken by nursing mothers, its fruits or seeds are used to thicken plant milk and to make vegetarian cheese [10].

Withania somnifera belongs to Solanaceae having a height from 0.5 upto 2 m, dispersed over desiccant regions of tropic and subtropic areas from the Canary Islands, Middle East, South Africa, Sri Lanka, India and upto China, Pakistan, Palestine, Egypt, Afghanistan, Eastern Africa, Jordan, Spain, Morocco, Congo and Madagascar. The mentioned regions represent extensive variant of soil, temperature, altitude and rainfall. In addition to the current explanation [11] of Ashwaganda Kaul, 2 spp of *Withania* are spread in Indian subcontinent [12]. *Withania somnifera* *coagulans* Dunal, a xerophytes plant spp, cultivates wild one in Baluchistan (Pakistan). It have been recorded from Sutlej-Valley, Indian Punjab however it did not recovered in these regions for many era. *Withania somnifera* Dunal is of extensive distribution all over north-west India, Bombay, Madhya Pradesh, Gujarat, Rajasthan, Western Uttar Pradesh and Punjab plains up-to mountain area of Punjab, Jammu and Himachal Pradesh in India the specie is cultivated as a medicinal crop [13]. The seeds are cultivated July before the oncoming of the rainy seasonal weather. It has been sown in gardens in the warmer climatic regions of Europe and occupied the status of wild weed in some parts of Australia [14]

1. MATERIALS AND METHODS

1.1. Chemical and Solvents

S.no	Chemicals
1	Carbon disulfide
2	Acetic Acid(CH ₃ COOH)
3	Ethanol/Methanol
4	D/H ₂ O
H5	Butanol
6	Sulfuric Acid (H ₂ SO ₄).
7	Chloroform(CH ₂ Cl ₂)
8	Acetone
9	Hexane
10	Liq Ammonia (NH ₃)

1.2. Plant Collection

The plant *Withania somnifera* was collected from the different areas of Bannu, KPK Pakistan, in 2021

1.3. Fractionation

The dried crude extract was subjected to fractionation. Various solvents were passed on it on the basis of increasing order of polarity. Following steps were followed during fractionation. The crude was dissolved in Methanol (the most polar org solvent) and then extracted with Hexane (the least polar solvent) by passing on it and then separated through separating funnel. The hexane fraction is.

f. Water fraction.

g. Crude fraction

2.4. . Screening Tests of Plant chemicals

Following phytochemical screening tests were performed for the detection of natural products present in the fruits of *Withania somnifera*.

- ✓ Alkaloids
- ✓ Tannins
- ✓ Saponins
- ✓ Sterols.
- ✓ Screening of Flavonoid
- ✓ Terpenoids
- ✓ Cardiac Glycosides
- ✓ Anthocynidins
- ✓ Anthraquinones
- ✓ Photobathnins

2. RESULTTS AND DISCUSSION

After polytochemical screening test different types of results were obtained, which are given below.

2.1. Saponins

The screening test performed for saponins given diverse fraction as given in the following results.

3.1.1. Butanol Fractions.

3.1.2. Ethyl Acetate Fraction

3.1.3. Hexane Fraction

3.1.4. Chloroform Fraction

3.1.5. Water Fraction

3.1.6. Crude Fraction

3.1.7. Defatted Ethanolic Fraction

3.2. Tanins

For various fraction of tanins screening test were performed which are given below.

3.2.1 Butanol Fraction

3.2.2. Ethyl Acetate Fraction

3.2.3. Hexane Fraction

3.2.4. Chloroform Fraction

3.2.5. Water Fraction

3.2.6. Crude Fraction

3.2.7. Defatted Fraction

3.3. Sterols

The screening test performed for the various fraction of sterols and obtained the following results.

3.3.1. Butanol Fraction

3.3.2. Ethyl Acetate Fraction

3.3.3. Hexane Fraction

3.3.4. Chloroform Fraction

3.3.5. Water Fraction

3.3.6. Crude Fraction

3.3.7. Defatted Fraction

3.4. Flavonoids

Wach extract (0.3g) was dissolved in the sodium hydeoxide (NaOH) and dilute Hydro Chloric acid (HCl) was added for detection of presence of flavonoids, following results were obtained in different fractions.

3.4.1. Butanol Fraction.

3.4.2. Ethyl Acetate Fraction

3.4.3. Hexane Fraction

3.4.4. Chloroform Fraction

3.4.5. Water Fraction

3.4.6. Crude Fraction

3.4.7. Defatted Fraction

3.5. Terpenoids

Chloroform (CH_3Cl_2) of 5ml was added to 0.8g of each extract along with 5ml of Cone H_2SO_4 . the following results were obtained from different fraction.

3.5.1. Butanol Fraction

3.5.2. Ethyl Acetate Fraction

3.5.3. Hexane Fraction

3.5.4. Chloroform Fraction

3.5.5. Water Fraction

3.5.6. Crude Fraction

3.5.7. Defatted Fraction

3.6. Cardiac Glycosides

Every isolate (0.7g) was diluted in D/H₂O and filtrated. The supernatants was heated in a 4 percent HCL solution. The presence of Photobathnins was confirmed by Red ppt.

3.6.1. Butanol Fraction

3.6.2. Ethyl Acetate Fraction

3.6.3. Hexane Fraction

3.6.4. Chloroform Fraction

3.6.5. Water Fraction

3.6.6. Crude Fraction

3.6.7. Defattd Fraction

3.7. Anthocynidins

HCl of 2ml was added on 0.7g of each content. The screening test for the presence of Anthocynidins was negative in some fractions except butanol and defatted. There was no red colour appear in some fractions except butanol and defatted.

3.8. Photobathnins

3.8.1 Butanol Fraction

3.8.2. Ethyl Acetate Fraction

3.8.3. Hexane Fraction

3.8.4. Chloroform Fraction

3.8.5. Water Fraction

3.8.6. Crude Fraction

3.8.7. Defattd Fraction

Table 1 Screening Test result for Phytochemical Detection in Various Fractions of Fruit extract of *Withania somnifera*

Name of Test	Butanol	Ethyl Acetate	Hexane	Chloroform	Water	Crude	Deffated
Tanins	×	×	×	×	×	×	×
Sterols	×	✓	×	✓	×	×	×
Flavonoids	✓	✓	✓	✓	✓	✓	✓
Anthocynidins	✓	×	×	×	×	×	✓
Photobathnins	×	×	×	×	×	×	×
Saponin test	×	×	×	×	✓	✓	✓
Cardiac-Glycoside	×	×	×	×	✓	×	✓
Terpenoid-test	✓	×	×	✓	×	✓	×

3.9. Antioxident Activity

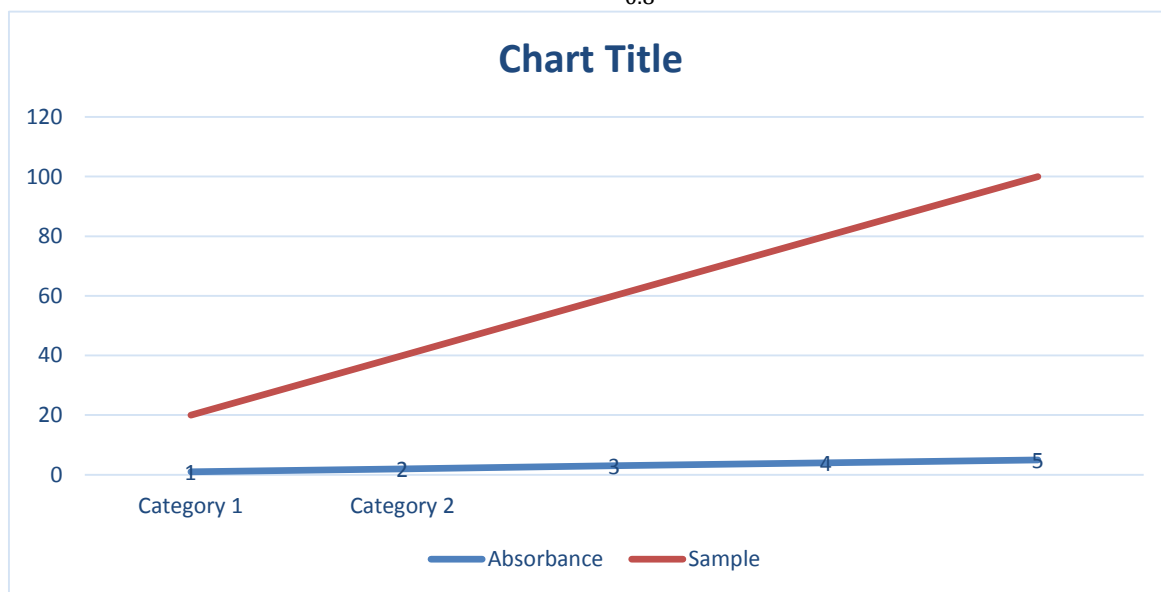
During the antioxidant activity following results was obtained from the different reading.

- 25 µl sample and 97 micro-liter DPPH solution was take incavit. The absorbance was 0.225
- 50 µl sample and 950 micro-liter DPPH solution formed and then the absorbance was 0.230.
- 75 µl sample and 925 micro-liter DPPH solution formed and then the absorbance was 0.233.
- 100 µl sample and 900 micro-liter DPPH solution formed then the absorbance was 0.292.

By applying formula to determine the % antioxidant activity

$$\frac{\text{Absorbance of DPPH} - \text{Absorbance of Sample}}{\text{Absorbance of DPPH}} \times 100$$

- For 25 µl sample % antioxidant activity $\frac{0.8-0.225}{0.8} \times 100 = 71.87 \%$
- For 50 µl sample the % antioxidant activity $\frac{0.8-0.230}{0.8} \times 100 = 71.25 \%$
- For 75 µl sample the % antioxidant activity $\frac{0.8-0.233}{0.8} \times 100 = 70.87 \%$
- For 100 µl sample the % antioxidant activity $\frac{0.8-0.292}{0.8} \times 100 = 63.5 \%$



Graph 1. Phytochemicals Absorbance Graph

The naturally vital chemical ingredients of *Withania somnifera* (WS) consist of alkaloids (isopelletierine, Anaferrine, cuseohygrine, anahygrine, etc.), steroidal lactones (withanolides, withaferin) and saponins [15]. Sitoinosides and acylsteryl glucosides in Ashwagandha are compounds resist stress. Biologically reactive compounds of Ashwagandha, for example the sitoinosides VII-X and Withaferin-A, presented to have noteworthy anti-stress activity in contraso severe models of experimental tress [16]. Many of its ingredients support immunomodulatory withasomniferin-A [17]. Chief constituents of *Withania somnifera* consist of steroidal lactones and alkaloids; these compounds are together termed as withanolide and are accountable for itsbiological effects. These withanolide have an ergostane framework with a C9-side chain in the form of a lactone ring [18] usually connected to the 'D' ring of the steroid(Figure 1-2).Some of the withanolide be present as glycosides formed with the hydroxyl group on the 'A' ring (e.g.withanosides-IV) or with the lactone (e.g.sitoinoside). on the basis of fundamental similarity of withanolides to the ginsenosides occurs in Panax ginseng, Ashwagandha is also called as the Indian Ginseng [19].

Withania somica has been reported (have anti-atesy, anti-nfanatoy and anti aging for in experimental animals and some clinicalconditions [20]. *Withania somnifera* have strong effect against cancer causing agents. Research on animal cell cultures has proven that some of the chemical constituents found in the herb reduces the ikensties of the nuclear factor kappa B and eliminates the intercellular cancer necrosis fiscor [21]. Ashwagandha has been reported to be beneficial against arthritis and other inflammatory, disorders.The steroidal amount naturally occurring in it is greater the prescribed anti-

inflammatory drugs [22]. The anti-aging activity of *Withania somnifera* is well established. In a double blind clinical trial *Ashwagandha* was evaluated for its anti-aging properties, 101 healthy people, about 53-62 years old persons were given the herb at a dosage of 4 grams daily for about 11 months. These people experienced noteworthy development in hemoglobin and hair melanin concentration and red blood cell count. Reduced blood cholesterol level and nail Ca^{+2} was preserved. About 70 percent of the research subjects were observed with advance in sexual activity [23]. Animal research using *Ashwagandha* have shown its influence upon the physiology of thyroid. Mice were given an extract of dehydrated *Withania* root orally every day for 30 days at a daily dosage of 1.4 grams per kg body weight. Massive gains in serum T4 levels were observed, revealing that the plant stimulates the glandular stage. Through its effect on cellular antioxidant systems, *Withania somnifera* can indirectly stimulate thyroid activity. These findings established that *Ashwagandha* can be a beneficial plant in the treatment of hypothyroidism. [24,25].

The biological activities shown by *Withania somnifera* are due to its primary and secondary metabolites. The chemical constituents of *Withania somnifera*, mainly the secondary metabolites is of great interest to scientific community. Phytochemical studies indicated that fruits of *Ashwagandha* have anti-inflammatory, antitumor, antioxidant, anti-stress, revitalizing and Immunomodulatory properties [26]. In addition it also has an impact on the cardiopulmonary, endocrine, and central nervous system [26]. For carbohydrates, glycosides, alkaloids, and fixed oil and fats when we do phytochemical analysis of this plant it showed positive results. Milk thickening characteristics are found in the fruit berries that has been used as ferment for cheese and in the preparation of vegetable residue [20]. The *withania somnifera* fruits are designated to be tranquilizer, emetic, blood cleanser and febrifuge and substitute, diuretic and bitter stimulant in digestive disorders as well as growing agent in children [21]. Owing to broad use of *withania somnifera* as a traditional medication for the cure of several diseases. From fruits of *withania somnifera* fixed oil have been extracted to check their biotic activities such as phytotoxicity, antibacterial, antifungal and cytotoxicity events. The results of current research was in agreement with the above mentioned work conducted at the Bannu University of Science and Technology, Khyber Pakhtunkhwa Pakistan. [27]

3. CONCLUSION

During the current study of *Withania somnifera* would be a best natural source of potent. This biologically important medicinal plant contains various natural products in all parts i.e.s in areal parts, flowers, leaves, stem and underground roots. The research project conducted for determining and exploration of different natural products by qualitative phytochemical screening test from various fractions. Current study supports that the plant contains many natural products in different fractions. The research confirmed important natural products are present in Hexane, Chloroform, ethyl acetate, butanol and water fraction and can be extracted easily by different methods.

REFERENCES

1. Kafaru, E Immense Help Formative Workshop. In: Essential Pharmacology, Istedn. Elizabeth Kafaru Publisher, Lagos, Nigeria (1994), 11-14.
2. Manna, A.; Albalka, M.E. *Spectrum J.* (2000), 7, 119-125

3. Shariff, Z.U. Morden Herbal Therapy for Common Ailments. Nature Pharmacy Series, Spectrum Book Limited, Ibadan, Nigeria in Association with Safari Book Export Limited, United Kingdom. (2001), Vol. 1, 9-4.
4. Girish, K.S.; Machiah, K.D.; Ushanandini, S.; Harish Kumar, K.; Nagaraju, S.; Gociindappa, M.; Vedavathi, M.; Kemparaju, K.J. Basic Microbiol. (2006), 46, 365-374
5. Dhuley JN. Effect of Ashwagandha on lipid peroxidation in Stress-induced animals. J Ethnopharmacol (1998) 60:173-178
6. Rege, N.N., Thatte, U. M., Dahanukar, S. A.; Adaptogenic properties of Six Rasayana herb used in Ayurvedic medicine. Phytother. Res (1999) 13:275-29
7. Bhattacharya, A., Ramanathan, M., Ghosal, S. and Bhattacharya, S.K., 2000. Effect of Withania somnifera glycowithanolides on iron-induced hepatotoxicity in rats. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 14(7), pp.568-570.
8. Bhattacharya, S.K., 1998, April. Adaptogenic activity of siotone, a herbal formulation against an unpredictable chronic stress induced physiological and behavioral perturbation in rats. In National conference on recent trends in Spice and Medicinal Plant Research.
9. Changhadi Govardhas Sharma- Ashwagandha (Indian ginseng) Rastanta Sar EvamSidhyaprayog Sangrah- Krishna Gopal Ayurvode Bhawan (Dharmarth Trust), Nagpur (1939), 743-744.
10. Facciolas; Comucopla, A Source Book of Edible Plants, Vista Kampong Publications.
11. Kaul, K.N., 1957. The origin, distribution and cultivation of Ashwagandha the so called Withania somnifera of Indian literature. In Symposium on the Utilization of Indian Medicinal Plants. Council of Scientific & Industrial Research, New Delhi (pp. 7-8).
12. Hooker, J>D, The flora of British India, London (1885).
13. Van Wyk, B.E., 2008. A broad review of commercially important southern African medicinal plants. Journal of ethnopharmacology, 119(3), pp.342-355.
14. Hepper, F.N., 1991. Old World Withania (Solanaceae): a taxonomic review and key to the species. loc. cit, pp.211-227.
15. Mishra, L.C., Singh, B.B. and Dagenais, S., 2000. Scientific basis for the therapeutic use of Withania somnifera (ashwagandha): a review. Alternative medicine review, 5(4), pp.334-346.
16. Ghosal, S., Lal, J., Srivastava, R., Bhattacharya, S.K., Upadhyay, S.N., Jaiswal, A.K. and Chattopadhyay, U., 1989. Immunomodulatory and CNS effects of sitoindosides IX and X, two new glycowithanolides from Withania somnifera. Phytotherapy Research, 3(5), pp.201-206.
17. Atta-ur-Rahman, Samina-Abbas, Dur-e-Shahwar, Jamal, S.A., Chaudhary, M.I. and Abbas.S New withanolides from Withania Spp. Journal od Natural Products, (1991), 56; 1000-1006.
18. Elsakka, M., Grigorescu, E., Stănescu, U., & Dorneanu, V. (1990). New data referring to chemistry of Withania somnifera species. Revista medico-chirurgicala a Societatii de Medici si Naturalisti din Iasi, 94(2), 385-387.
19. Grandhi, Anuradha, A. M. Mujumdar, and Bhushan Patwardhan. "A comparative pharmacological investigation of Ashwagandha and Ginseng." Journal of ethnopharmacology 44.3 (1994): 131-135.
20. Bhattacharya Sk; Muruganandam AV; PharmacolBiochemBehav, (2003), 547-555.
21. Bhattacharya, S. K., et al. "Anxiolytic-antidepressant activity of Withania somnifera glycowithanolides: an experimental study." Phytomedicine 7.6 (2000): 463-469.

22. Bhattacharya, S. K., et al. "Antioxidant activity of Bacopa monniera in rat frontal cortex, striatum and hippocampus." *Phytotherapy Research* 14.3 (2000): 174-179.
23. Bone K; *Clinical Applications of Ayurvedic and Chinese Herbs*. Queensland Australia.
24. Prakash, Jai, Suresh Kumar Gupta, and Amit Kumar Dinda. "Withania somnifera root extract prevents DMBA-induced squamous cell carcinoma of skin in Swiss albino mice." *Nutrition and cancer* 42.1 (2002): 91-97.
25. Jayaprakasam, Bolleddula, et al. "Growth inhibition of human tumor cell lines by withanolides from Withania somnifera leaves." *Life sciences* 74.1 (2003): 125-132.
26. Mishra, Lakshmi-Chandra, Betsy B. Singh, and Simon Dagenais. "Scientific basis for the therapeutic use of Withania somnifera (ashwagandha): a review." *Alternative medicine review* 5.4 (2000): 334-346.
27. Christina, A. J. M., et al. "Anticarcinogenic activity of Withania somnifera Dunal against Dalton's ascitic lymphoma." *Journal of ethnopharmacology* 93.2-3 (2004): 359-361.