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PHYTOCHEMICAL SCREENING AND CHARACTERIZATION OF WITHANIA SOMNIFERA FOR THEIR ANTIMICROBIAL AND ANTIOXIDANTACTIVITY

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ABSTRACT: ***Ayurveda is one of the traditional medicinal systems of Indian culture. The philosophy behind Ayurveda is preventing unnecessary suffering and living a long healthy life. Ayurveda involves the use of natural elements to eliminate the root cause of a disease by restoring balance and at the same time creating a healthy life-style to prevent the recurrence of imbalance. Herbal medicines have existed world-wide with long recorded history. World Health Organization (WHO) have estimated that 80% of the world's inhabitants still rely on traditional medicines for their health care. India is well-known to be one of the major biodiversity centre with about 45,000 plant species, including 15,000 medicinal plants. The concept of polyherbalism is to achieve greater therapeutic efficacy. The active phytochemical constituents of individual plants are insufficient to achieve the desirable therapeutic effects. When combining this research mainly focuses on the importance of polyherbalism and its clinical significance. For this study medicinal plant Withaniasomniferahave been taken and extracted for their study of anti-bacterial and anti-oxidant activity. The phytochemical compounds were screened by qualitative analysis method and the detected phytochemicals are tannins, saponins, alkaloids, phenols, terpenoids, flavonoids. Thedifferentsolventssuchasmethanol,petroleumether,chloroformandaqueouswereusedto extract the bioactive compounds from various parts of the selected medicinal plants. The anti-bacterial activity were demonstrated against the bacterial strains like Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosaby disc-diffusion method. The anti-oxidant activity was evaluated by DPPH radical scavenging method. The multiple herbs in a particular ratio, it will result a better therapeutic effect and reduced the toxicity.***

Keywords: Polyherbal Formulation, Phytochemical Screening, Anti-Microbial Activity, Anti- Oxidant Activity, DPPH Method, Phytotherapy, Traditional Medicine.

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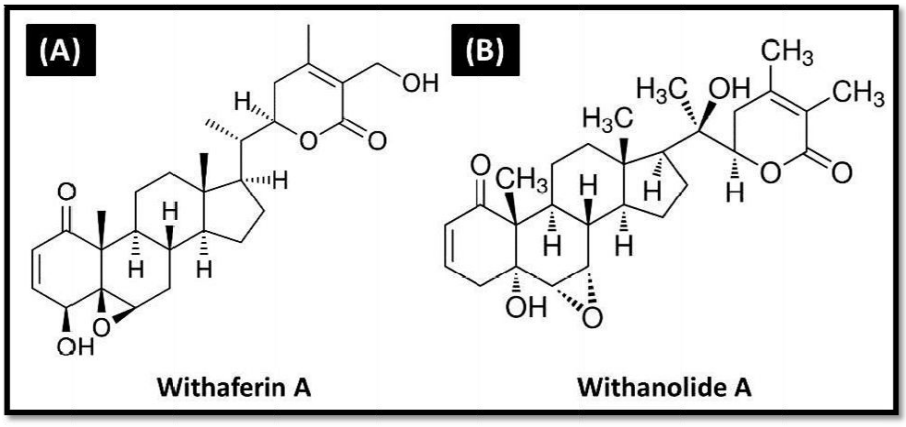
I. INTRODUCTION

*Withaniasomnifera*is also known as Ashwagandha, Indian ginseng and winter cherry, it has been an important herb in the Ayurvedic and indigenous medical systems for over 3000 years. The roots of the plant are categorised as rasayanas, which are reputed to promote health and longevity by augmenting defence against disease, arresting the ageing process, revitalising the body in debilitated conditions, increasing the capability of the individual to resist adverse environmental factors and by creating a sense of mental wellbeing. It is in use for a very long time for all age groups and both sexes and even during pregnancy without any side effects. Historically, the plant has been used as an antioxidant, adaptogen, aphrodisiac, liver tonic, anti- inflammatory agent, astringent and more recently to treat ulcers, bacterial infection, venom toxins and senile dementia. Clinical trials and animal research support the use of *Withaniasomnifera*for anxiety, cognitive and neurological disorders, inflammation, hyperlipidemia and Parkinson's disease. Recently WS is also used to inhibit the development of tolerance and dependenceon chronic use of various psychotropic drugs. A number of new antibiotics have been produced by the pharmacological industries in the last three decades, resistanceto these drug hasincreasedinmicroorganisms.Bacteriagenerallyhavethegeneticcapacitytotransmitand acquire drug resistance. Most synthetic drugs also protect against damage to oxidation butthese drugshaveadversesideeffects.Therefore,actionsmustbetakentodecreaseteseproblemsand oneofthewaystooutdothisproblemisbyusingplantswhichhaveanexcellentsourceof medicine,naturalanti-oxidantsandfoodsupplements.Recently,countlessnaturalcompounds withanti-microbialandanti-oxidantpropertieshavebeenisolatedfromdifferentplantmaterials. Morethan80%oftheworld'spopulation,accordingtotheWorldHealthOrganization(WHO), relies on traditional medicine for their primary healthcare needs.

Chemical Constituents:



Taxonomical Classification

- Kingdom : Plantae(Plants)
- Subkingdom : Tracheaobionta (VascularPlant)

- Superdivision : Spermatophyta (SeedPlants)
- Division : Magnoliophyta (FloweringPlants)
- Class : Magnoliopsida(Dicotyledone)
- Subclass : Asteridae
- Order : Solanales
- Family : Solanaceae
- Genus : *Withania*
- Species : *Withaniasomnifera*

Withaniasomnifera(Ashwagandha)isanerect,sweet,astringent,evergreenshrub.Itmainly poseonthereproductiveandnervoussystems.Ithassedative,revitalizeandaphrodisiaceffects. Itisprescribedincaseoffatigueorexhautionwhereitisreportedtopromotestrength,vigorand vitalityandactsasnature'sbestadaptogen(anadaptogenstrengthentheimmunesystem,protects againstmentalandphysicalfatigue,fightsstress,tensionandregularizesallbodyfunctions).In additiontoitstherootsandleavesoftheplantareusedtraditionallyintheformofpowder, decoction,oiletc.thesehavebeenusedinconventionalmedicineagainstgeneralenervation, hypertension, inflammations, asthma, cancer, tuberculosis, tumors, rheumatism, psoriasis,senility, smallpox, sores, syphilis, scabies, ringworm, typhoid, uterosis and wounds. It possesses anti-inflammatory, anti-tumor, anti-stress, anti-oxidant, immunomodulatory, hemopoietic and rejuvenating properties.

II. MATERIALS & METHODS

- **Microorganisms:** *E.coli*Culture, *Staphylococcus aureus*, MTCC (Microbial type culture collection)
- **Glasswares:** Petri plates, Pipettes (1ml & 2ml), Measuring cylinder, Flask, Beaker, Jam bottles, Glass rod, Volumetric Flask, Test tubes, Conical Flask,Funnel.
- **Miscellaneous:** Cotton, Inoculation loop, Whattmann filter paper, Centrifuge tubes, Micropipettes, Disk, Tips, Forceps, Hi Media antibiotic Zone Scale (for Zone measurement), Dropper, Aluminum foil, Rubber band, Glossy papers, Pipette bulbs, test tube stand, Wash Water, Glass slide, Icepack.
- **Chemicals Required:** 95% ethanol, Distilled water, Nutrient Broth, Agar, Nutrient Agar Media, Culture, Herbal Drug powder (Ashwagandha), Chloroform, Methanol, Petroleum ether, Fehling solution A & B, Ferric chloride, Mayer's reagent (Mercuric Chloride, Potassium Iodide), Ninhydrin solution, DPPH (Diphenylpicryl Hydrazine), Sodium Hydroxide, Biuret Reagent, Conc. Sulphuric Acid, Acetic Acid, Dilute Hydrochloric Acid, DiclofenacSodium.

- **Instruments:**
  1) Soxhlet Assembly (J-Sil, 50/42, Borosil glass) - For extracting the phytochemicals of powdered drug with the help ofsolvents.
  2) Vacuum Rotary Evaporator (Scientech) - For evaporating the phytochemicals present in theextraction.
  3) Digital Balance (Denver, Germany) - For weighing chemicals in microquantities.
  4) Hot Air Oven (Scientech, 325 L) - For sterilizing the glass wares afterwashing.
  5) Laminar Air Flow Chamber Horizontal - For maintenance of asepticcondition.
  6) Incubator (Scientech) - For the growth of themicroorganism.
  7) Cyclo Mixer (REMI) - For mixing thesuspensions.
  8) Antibiotic Zone Scale Laboratories Ltd - For the measurement of zone ofinhibition

III. SAMPLE COLLECTION:

The whole plant was collected from Govt. Nursery of Ujjain, M.P. India

Preparation of Plant Extracts

200 ml of solvent (Chloroform, Methanol, Petroleum ether, and aqueous) was taken ina round bottom flask.Then20 gmofthe drugpowder was weighedin a digital weighingmachine and wrapped in a filter paperto make athimble. It was thenplacedinthe centralcompartment&itwasheatedatatemperaturerangebetween50^{0}C-60^{0}Cinaheatingmantle.

After heating the vapour passes through the side arm up into the reflux condenser. Herethevapour condenses, liquefies & drips into the thimble containing the material to be extracted. The warm solvent percolates through the material & the wall of the thimble & the extract gradually collects in the central compartment. Once the height of the extract reaches the top of the siphon, the entire liquid in the central compartment flows through this & back into the lower round bottomed flask. Then the process is further repeated as required. In this method the extract gets collected in the lower vessel and gradually becomes more & more concentrated. When the drug powder was completely extracted, the solvent collected in the middle compartment displayed transparent colour. Assuming that there are novolatile substances present, the vapourisation from the heated extract is pure solvent in the vapour form & so the liquid dripped into the material from the condenser is essentially pure solvent, though derived from the extract, thus although a relatively small volume of solvent is needed. The effective volume of solvent used for the extraction is proportional to the time for which the process is allowed to continue. The extraction process was repeated for Chloroform,

Methanol and Petroleumether.

Phytochemical Test	*Withaniasomnifera*(Ashwagandha)			
	Chloroform	Methanol	Petroleum Ether	Aqueous
Alkaliods	+	+	+	-
Flavonoids	+	+	+	-
Tannins	+	+	+	-
Phenols	+	+	+	-
Terpenoids	+	+	+	-
Saponins	-	+	-	+

Table 1: Phytochemical Analysis Test Chart of *Withaniasomnifera*
(+) --- Positive
(-) --- Negative

Anti-Bacterial Activity By Disc Diffusion Method

Preparation ofInoculum

*E.coli*and *S.aureus*strains were used. 60 ml of Nutrient broth was prepared in 100 ml conical flask. It was sterilized & then inoculated with inoculum with the help of sterile loop in laminar air flow from preserved slants. They were then kept in incubator at 37^0C for sufficient period of time for organism to grow.

Disc Diffusion Method

After solidification the disc of whatmann filter paper imbibed with 20 µl plant extracts were carefully placed with the help of forceps at the centre of the petri dish and then kept in incubator for 24hrs.

Measurement of Zones

With the help of antibiotic zone scale the zone of inhibition (ZOI) were measured.

ANTIOXIDANT ACTIVITY

Preparation of Reagent

DPPHReagent---------- 2 mg of DPPH was taken & dissolves in 100 ml ofMethanol.

AscorbicAcid---------- 0.2 gm of Ascic acid iorbn 100 ml of distilledwater

Method

11 clean test tubes were taken and ascorbic acid solution was added to each of the test tubes in an increasingamountfrom0.2,0.4,Theeleventhtesttubewaskeptblankwithnoascorbicacid.Then methanol was added to make the final volume to 2 ml. Then 0.5 ml of DPPH solution was added to each of the test tubes. The test tubes were allowed to stand for the reaction to occur for 10 min in dark conditions. Finally the readings were noted down by the help of UV VIS SHIMADZU 1800 Spectrophotometer at 517nm. In case of extracts obtained from herbal sample same procedure was used. 20 µl of the samples were taken & volume was made to 2 ml with methanol. 0.5 ml of DPPH solution was added to each of the test tubes and it was allowed to stand for reaction for 10 min in dark conditions. Reading was noted down onUV VIS

SHIMADZU 1800 Spectrophotometer at 517nm. Determination of percentage inhibition of DPPH Activity by using following formula:

% Inhibition of DPPH Activity = A-B/A *100

Where,

A = Optical Density (O.D.) of the blank B = Optical Density (O.D.) of thesample

IV. RESULTS & DISCUSSION

Colour of Successive Extracts

Sl. No.	Name of Reagent	Name of Drug	Colour of Extract
01.	Chloroform	*Withaniasomnifera*	Pale Green
02.	Petroleum Ether	*Withaniasomnifera*	Colourless

03.	Methanol	*Withaniasomnifera*	Brown
04.	Aqueous	*Withaniasomnifera*	Light Yellow

Table 2: Anti-Bacterial Activity of Drug Extract From Soxhlate Extraction Method

Chloroform Extract

Sl. No.	Name of the Drug	Microorganism	Zone of Inhibition (in mm)
01.	*Withaniasomnifera*	*E. coli*	06 mm
02.	*Withaniasomnifera*	*S. aureus*	12 mm

Table 3: Anti-Bacterial Activity of Chloroform Extract of *Withaniasomnifera*

Petroleum Ether Extract

Sl. No.	Name of the Drug	Microorganism	Zone of Inhibition (in mm)
01.	*Withaniasomnifera*	*E. coli*	05 mm
02.	*Withaniasomnifera*	*S. aureus*	NO ZOI

Table 4: Anti-Bacterial Activity of Petroleum Ether Extract of *Withaniasomnifera*

Methanol Extract

Sl. No.	Name of the Drug	Microorganism	Zone of Inhibition (in mm)
01.	*Withaniasomnifera*	*E. coli*	18.66 mm

02.	*Withaniasomnifera*	*S. aureus*	14.33 mm

Table 5: Anti-Bacterial Activity of Methanol Extract of *Withaniasomnifera*

Aqueous Extract

Sl. No.	Name of the Drug	Microorganism	Zone of Inhibition (in mm)
01.	*Withaniasomnifera*	*E. coli*	08 mm
02.	*Withaniasomnifera*	*S. aureus*	No ZOI

Table 6: Anti-Bacterial Activity of Aqueous Extract of *Withaniasomnifera*

ZOI - (Zone of Inhibition)

Sl. No.	Microorganism	Zone of Inhibition (mm)	
		Penicillin G	Oflaxacin
01.	*E. coli*	17 mm	18 mm
02.	S. *aureus*	16 mm	19 mm

Table 7: Anti-Bacterial Activity of Some Standard Antibiotics

DISCUSSION

The powdered drug was subjected to successive extraction protocol soxhalation. The extract so obtained was tested for the presence of phytochemical like alkaloid, carbohydrate, amino acid, Glycosides, Phenolic compounds and Tannins. The anti-bacterial activity of the powder extract was done with Chloroform, methanol and petroleum ether. The results indicate that the anti- microbial activity of the methanolic extract of Ashwagandha was comparable with

standardantibiotic. This shows the Ashwagandha has an anti-bacterial activity and this may be due to the extracted phytochemicals in methanolic extract. According to Mirjalili et.al (2009) the important compounds withferin and withanolides were isolated from the methanolic extract of the roots of the *Withaniasomnifera*. But further chemical characterization is needed to confirm the molecule responsible for the activity. The anti-bacterial activity of this herbal formulation was comparable with standard antibiotics like Penicillin G andOflaxacin.

Anti-Oxidant Activity of *WithaniaSomnifera*

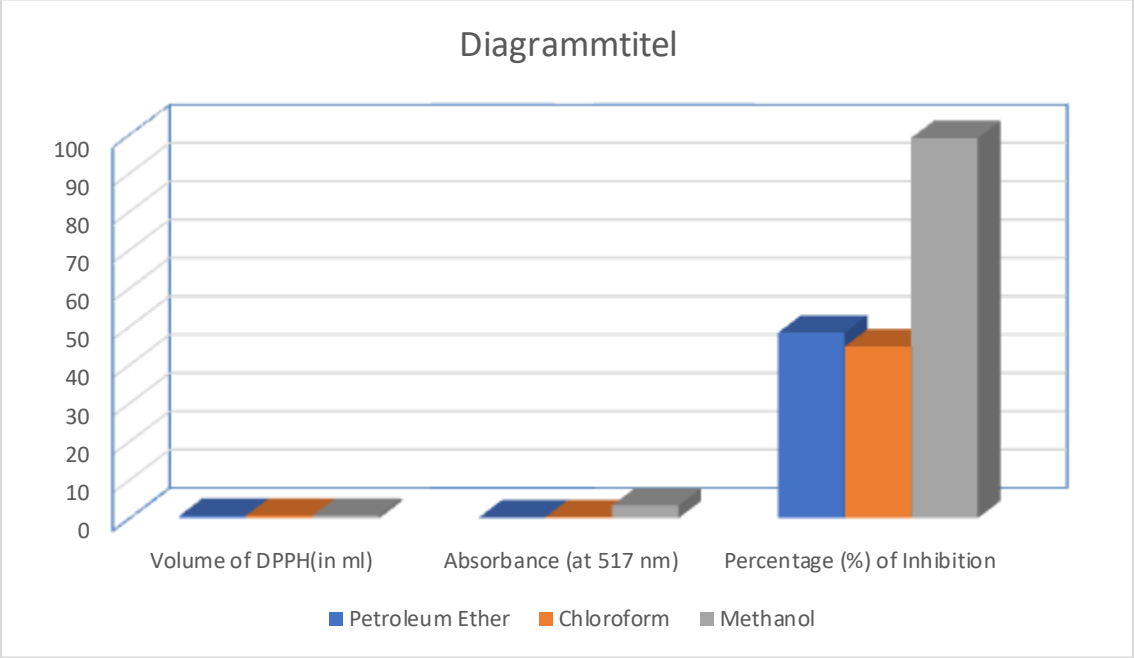
Phytochemical screening reveals that the major constituents of Ashwagandha extract are phenolic compound, glycosides, alkaloid and flavanoid. Among these phenolic compounds which may be responsible for the activities ofanti-oxidant.

DPPH Radical Scavenging Activity

Ashwagandha had significant scavenging effect on the DPPH free radical which increased with increasing concentration. The scavenging effect of sample was lower than that of Ascorbic acid.

Sl. No.	Volume of Sample (200µl)	Volume of Methanol (in ml)	Volume of DPPH (inml)	Absorbance (at 517 nm)	Percentage (%) of Inhibition
01.	Petroleum Ether	3 ml	0.7	0.213	48.4
02.	Chloroform	3 ml	0.7	0.300	44.8
03.	Methanol	3 ml	0.7	3.315	99.2

Table 8: Observation Table of DPPH Method for Determining the Percentage of Inhibition



Radical Scavenging Activity of *withaniasomnifera*

V. CONCLUSION

The results of this study clearly indicate that Ashwagandha have high anti-oxidant activity and radical scavenging activity against various anti-oxidant systems in vitro. These assays have important applications for the food and pharmaceutical industry. Moreover, Ashwagandha can be used as an easily accessible source of natural antioxidants and as a possible food supplement. In our present study we conclude that Ashwagandha has good anti-oxidant property and could be attributed to the presence of flavonoids, alkaloids, tannins, saponin and phenolic compounds. It was already reported that naturally occurring phenolic compounds have free radical scavenging property.

VI. FUTURE PROSPECTS

The Herbal formulations have its own importance and advantages as compare to any other forms of medicines. As discussed in the present research the herbal formulations are free from any undesirable side effects and more or less they are non habit forming. The Indian climate favours the growth of many rare varieties of medicinal Plants. But the need of the hour is, these plants should be identified and much extensive research should be done on it so that new Drug discovery can be made to cure many threatful diseases. Many research organizations and Industries are pursuing research on exploring the flora like CIMAP, Himalayan Drugs etc. and many success stories are daily published. But the research should be carried out in a large scale and should be region specific so that new formulations can be prepared. Much work is also going on Polyherbal Formulation, in which many herbal drugs are scientifically mixed to get the synergistic effect.

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