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ESTABLISHING BIOTECHNOLOGICAL APPROACHES TO PRODUCES SECONDARY METABOLITES FROM EXTRACT OF *WITHANIA SOMNIFERA*

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Abstract

The Indian Himalayan region (BHR), one of the richest reservoirs of biological diversity in the world, is undergoing irrational extraction of wild, medicinal herbs, thus endangering many of its high value gene stock. *Withonia somnifera L*. (Dunal) is a member of solanaceae, also known for thousands of years by Ayurvedic practitioners. *Withania somnifera* root contains flavonoids, alkaloids, steroid and many active functional ingredients. *Withania somnifera* consists of very high concentration of secondary metabolites that can be also known as bioreactors like steroidal lactones, alkaloids and flavonoides, which have effective properties and they used in ninety commercially Ayurvedic formulations. *Withania sominfera* are propagated in northern western region of Madhaya Pradesh in India, on about 400 ha. But the risks of fungal infections are very high in these plants. *Withania sominifera* having small white flowers mainly in rainy and winter seasons that can be develop into fruit during the winter seasons. Plants products can be obtained from the roots, leaves, and branches, by using many different biological techniques. *Withania* which is also known as Ashwagandha having effective property can also used in blends and supplements which are designed to show many multiple effects. It is described as an herbal tonic and health food in Vedas and considered as 'Indian Ginseng' in traditional Indian system of medicine.

Keywords: Indian Himalayan region, *Withania*, Madhya Pradesh, Biological techniques, Traditional Indian system of medicine.

Introduction

Biotechnological approaches in medical field give offers and many uncommon, very important opportunities and protocol for several techniques to propagate, improve, conserve and utilize mineral elements medicinally (Lohar DR *et al* 1992) in a better and improve fashion. Due to totipotency (Das *et al* 2011) nature of plant cell, plant tissue culture (Kulkarni *et al* 1999) is one of the best method to produces large number of plants in very short time in a very small area. It is one of the best method to developed pathogen-free (Rajendran & Ramakrishnan 2009) stocks of plant, raising the formation of uniform clones (similar type of plants) from highly specific heterozygous plants. It also involve in the monitoring of production of natural useful products (Marderosion 2001) in lab (*in vitro),* inducing and propagating plants with altered genotypes and pushed this science to new development in modern technology by providing the base for new biotechnology (Kulkarni *et al* 1999).

Material and Methods

Phyto-chemical screening

The leaf, stem and root extract of *Withania somnifera* were analyzed for the presence of glycosides, steroids, phenolic compounds and flavonoids. One gram of sample was weighed and dissolved with various solvents such as ethanol, methanol and water. Then the sample was saturated to allowed to stay overnight for 24 hours. After overnight incubation with continuous shaking the sample extract was filtered by Whatman filter paper and the filtrate was centrifuged at 10,000 to 25,000 rpm for 10 minutes, and the supernatant was used for Phyto-chemical screening (Soni *et al* 2012).

Test for Glycosides

About 1ml glacial acetic acid mixed with 1 ml of extract was containing one limited drop of $FeCl_3$ solution and a brown ring obtained in solution when this was subjected with 1 ml of concentrated sulphuric acid. Presence of glycosides (Matsuda *et al* 2001) indicated by the formation a brown ring.

Test for Steroids

To the 0.5 ml of filtrate, 2ml acetic anhydride was added followed by an addition of 3ml concentrated sulphuric acid. Presence of Steroids (Mishra *et al* 2005) indicated by the formation of blue green ring.

Test for Phenolics

To the 2ml of *Withania* liquid extract, added with 1ml of 1% ferric chloride blue or green colour indicates the presence of phenolics.

Test for Flavonoids

A few limited drops of dilute NaOH were added to one ml of the *Withania* extract, yellow colour was produced in the extract, they become colour-less by addition of few certain drops of dilute acid indicates the occurance of flavonoids.

Test of phyto-chemicals

Root, stem and leaf of *W. somnifera* exhibited differences in the presence of secondary cell metabolites. Different plant parts were extracted with ethanol and methanol and the results were compared in the Table-4.3 and 4.4

Flavonoids, glycosides (Matsuda *et al* 2001) and phenols were present in leaf while steroid was present in both root and stem when extracted with ethanol.

When extracted with methanol flavonoids, steroids, glycosides and phenols were present in leaf, steroid was present in roots and glycosides and phenols were present in stem. Preliminary phyto-chemical screening (Soni *et al* 2012) of plant extract has been reported in several medicinal plants (Van *et al* 2000). In the present study, the different parts of the *Withania somnifera* contain flavonoides, glycosides, steroids and phenolic compounds were analyzed.

Results and Conclusion

Table 4.1: Macroscopic identification of leaf

S. No.	Important Characteristic	Macroscopic Observation
1	Taste	Bitter
2	Size	6-8 cm. long, 2-4cm. wide
3	Colour	Green
4	Odour	Characteristic
5	Base	Asymmetric/unequal
6	Apex	Subacute
7	Shape	Ovate
8	Margin	Entire
9	Venation	Alternate

Table 4.2: Standardization of powdered leaf (leaf extract)

S. No.	Parameter	Percentage
1	Moisture content	5%
2	Alcohol Soluble Extractive (90%)	18.50%
3	Acid Insoluble Ash	1.5%, 2.6%
4	Water Soluble Extractive	19.52%
5	Total Ash	5.29%, 5.89%

Table 4.3 : Extract solution, colour, consistency and percentage yield of *Withania's* extracts

S. No.	Extracts	Colour	Consistency	% extract yield (w/w)
1	Ethanolic	Brownish green	Solid	10.32
2	Hydroalcoholic	Brownish green	Solid	20.02
3	Chloroform	Dark green	Solid	4.36
4	Aqueous	Brownish green	Solid	09.14
5	Pet. ether	Dark green	Semisolid	1.30

Table 4.4 : Preliminary qualitative chemical evaluation and identification of plant extracts

S. No	Tests	Chloroform extract	Ethanolic extract	Petroleum ether extract	Hydroalcohlic extract	Aqueous extract
1	Glycosides	-	+	-	+	+
2	Saponins	-	+	-	+	+
3	Proteins and amino acids	-	+	-	+	+
4	Flavonoids	-	+	-	+	+
5	Volatile oil	-	-	-	-	-
6	Alkaloids	+	+	-	+	+
7	Fats and fixed oil	-	-	+	-	-
8	Carbohydrates	-	-	-	+	+
9	Tannins	-	+	-	+	+

Table 4.5 : Glucose content, Bitterness value

S. No	Glucose content	Bitterness value
1	0.015mg/gm	846 unit/gm

Table 4.6 :identification of Rf value through TLC of prepared extract

S. No.	Solvent system	Extract	No. of spots	Rf Value
1	Toluene:Ethylacetate (80:20)	Ethanolic	3	0.05 0.22 0.41
2	Pet.ether:Acetone (80:20)	Pet. Ether	6	0.09 0.18 0.22 0.30 0.42 0.97
3	Toluene : Ethylacetate : Aceticacid (60 : 38 : 2)	Hydroalcoholic	3	0.29 0.57 0.65
4	Chloroform:methanol (70:40)	Chloroform	4	0.07 0.16 0.21 0.48
5	Acetonitrile : Water (70:30)	Aqueous	2	0.36 0.86

Several neurodegenerative disorders successfully treated by several components obtain by *Withania* plant extract such as Parkinson's disease, alzheimer's disease, Amyotrophic lateral sclerosis and Huntington's disease (Chiti and Dobson 2006) and Creutzfeldt-Jakob disease (CJD) (Chakrabarti *et al* 2009) are some of the well known age related disorders. Despite the great number of ongoing investigations, neurodegenerative disorders remain incurable. The drugs obtain from *Withania*, currently available for dementia, such as donepezil, an acetylcholine inhibitor are effectious in the temporary treatment and curing of memory dysfunction or memory loss, but the drug from *Withania* do not prevent or reverse the neurodegeneracy (Elizabeth *et al* 2007). Reviews on the multiple and varied plant species of the natural molecules are available (Campos *et al* 2010).

The plant is being exploited for preparation of over 200 formulations used in the treatment of various physiological disorders (Antonisamy and Manickam 1999). It is used therapeutically as an adaptogen for patients with nervous exhaustion, insomnia, and debility due to stress and as an immune stimulant in patients with low white blood cell counts (Ven *et al* 2010). Over exploitation of plants and the reproductive failure associated with vulnerable to complete extinction (Antonisamy and Manickam 1999). Phytochemical analysis of different part of the plant was performed and the results were compared. The different parts of the *Withania somnifera* contain flavonoides, glycosides, steroids and phenolic compounds were analysed. Rapid multiplication approach could be a viable option in domestication and commercial cultivation of *Withania somnifera*.

References

Antonisamy R and Manikam VS 1999 Conservation through micro-propagation and restoration of selected rare and endangered medicinal plants of South India. XVI Int. Bot. Cong. held on 1-7 Aug. (Saint Louis, Missouri, USA)

Campos HC, da Rocha MD, Vie gas FP, Nicastro PC, Fossaluzza PC, Fraga CA, Barreiro EJ and Viegas C 2010 CNS Neuro. Disord. Drug Target

Chakrabarti O, Ashok A and Hegde RS 2009 Science. 34, 287-95

Chiti F and Dobson CM 2006 Annu Rev Biochem., 75, 333-366

Das A; Datta AK and Banerjee D 2011 Organogenic Totipotency In In Vitro Culture of Two Elite Genotypes (Poshita And Jawahar 22) of Withania Somnifera (L.) Dunal And Quantification of Bio-Active Compounds Datta Animesh Kumar et al / IJRAP , 2 (4) 1175-1179

Elizabeth H; Kristy B and Alvin VT 2007 Galantamine and Donepezil Attenuate Pharmacologically Induced Deficits in Prepulse Inhibition in Rats, 52(2), 542-551

Kulkarni AA, Thengane SR, Krishnamurthy KV 1999 De novo regeneration of various explants of *Withania somnifera* (L.) Dunal. In Plant Tissue Culture and Biotechnology. Emerging Trends (Ed. Kavikishor P.B.) Universities press, India: 180-186

Lohar DR; Chaturvedi D and Varma PN 1992 Mineral elements of a few medicinally important plants. Ind. Drugs. 29, 271-273

Marderosion AD 2001 The Review of Natural Products, Facts and Comparisons; St. Louis, MI, USA, 2001; pp 630-632

Matsuda H, Murakami T, Kishi A and Yoshikawa M 2001 Structures of withanosides I, II, III, IV, V, VI and VII, new withanolides glycosides, from the roots of Indian *Withania somnifera* Dunal and inhibitory activity for tachyphylaxix to clonidine in isolated guinea-pig ileum. Bioorg Med Chem 9: 1499–1507

Mishra L, Lal P, Sangwan RS, Sangwan NS, Uniyal GC and Tuli R 2005 Unusually sulphated oxygenated steroids from *Withania somnifera*. Phytochemistry 66, 2702–2707

Rajendran NK and Ramakrishnan J 2009 *In vitro* evaluation of antimicrobial activity of crude extracts of medicinal plants against multi drug resistant pathogens. Biyoloji Bilimleri Araptyrma Dergisi 2: 97-101

Soni P, Bahadur AN, Tiwari U And Kanungo VK 2012 *In vitro* multiplication and phyto-chemical analysis of a valuable medicinal plant *Withania somnifera* L. Dunal Indian J. Innovations Dev., *Vol*. 1, No. 4

Van WB, Oudtshoorn BV and Gericke N 2000 Medicinal plants of South Africa: Briza publications. 2000; pp 274