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Phytochemical Screening of Various Extracts of Root of Withania Somnifera (L) Dunal

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Abstract

Withania somnifera (L) Dunal (Solanaceae), commonly known as Aswagandha, is one of the most valued medicinal plants with a number of pharmaceutical applications. The roots are the main portion of the plant used in herbal medicine. Root extracts of W. somnifera are commonly used as a remedy for variety of ailments and a general tonic for over all health and longevity in the Traditional medicine system. The aim of the study was to investigate the secondary metabolites of various extracts of root of W. somnifera and quantification of some of the active constituents like alkaloids, flavonoids, saponins and volatile oil according to standard procedures. The preliminary phytochemical screening of cold and hot ethanol, methanol and aqueous extracts showed the presence of alkaloids, saponins, flavonoids, steroids, tannins, proteins, reducing sugar and coumarins and absence of quinones or anthraquinones. The total alkaloid, flavonoid contents were found to be 0.81 ± 0.01 %, 14.43 ± 0.40 % and total saponin content was (Foaming Index) FI < 100 respectively. The considerable amount of volatile oil was not determined in fresh root of W. somnifera. The findings are consistent with the presence of biologically active constituents in the polar extracts of W. somnifera and may provide helpful in authentication and identification of this plant.

Keywords: Different extracts, phytochemical screening, root, Withania somnifera

INTRODUCTION

Medicinal herbs are making a tremendous revival all over the globe. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Over three quarters of the world population relies mainly on plants and plants extracts for health care (Lakshmi et al., 2011). In the Traditional system of medicine, which dates back many centuries, uses many herbal extracts to cure a variety of diseases including carcinoma (Singh et al., 2005). One such popularly used plant that is reported to have anti-inflammatory, anti arthritic, antitumor, antioxidant, immunomodulatory, and hepatoprotective effects is Withania somnifera Dunal, which is commonly known as 'Ashwagandha' (Al-Hindawi et al., 1989; Mohammed et al., 1996; Rasool et al., 2000, Marie Winters, 2004; Subramanian et al., 2008 and Bhattacharya et al., 2008). It is useful in stress, strain, fatigue, pain, skin disease, diabetes, gastro intestinal disease, rheumatoid arthritis, and epilepsy (Kirtikar & Basu, 1935;

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Nadharni, 2002 & Sandhu et al., 2010). Due to its wide therapeutic importance it is worthwhile to obtain various qualitative and quantitative standards of drug to prevent its adulteration.

Although the phytochemical screening of this plant already published, this study is presented to compare on its phytochemical constituents in various extracts, which were performed by different extraction methods. Quantification of some of the active constituents like alkaloids, flavonoids, saponins and volatile oil were also carried out according to standard procedures. Thus, the present study deals with investigate the secondary metabolites of various extracts of root of W. somnifera.

MATERIALS AND METHODS

Plant materials and Chemicals: W. somnifera roots were purchased from a reputed vendor of herbal material (M. S. Marunthakam) in Jaffna District. The botanical identity of the plant was confirmed by the Botanist at Bandaranayaka, Memorial Ayurvedic Research Institute (BMARI), Navinna, Maharagma, Sri Lanka (Voucher specimen No. 2553).

All the reagents and chemicals used were procured from Institute of Industrial Technology {ITI}, Colombo- 07 and of analytical grade.

Preparation of Test material: The purchased W. somnifera roots were cut in to small pieces and gently boiled with cow's milk (1:1) for purification. These roots were air-dried thoroughly under shade (at room temperature) for 2-3 weeks to avoid direct loss of phytoconstituents from sunlight. The shade dried materials were powdered using the pulverizer and sieved up to 80 meshes. It was then homogenized to fine powder and stored in air-tight bottle for future use.

Preparation of the plant extracts: Hot extraction: A total of 10gm of powdered sample was taken and mixed with 50 ml distilled water in a round bottom flask and gentle refluxed for 1½ hour separately. The residue was removed by filtration through Whatmann No:1 filter paper and the aqueous extract was concentrated used on a Rotary evaporator (Buchi) for just as long as was required to remove the solvent, and re-dissolved the residue in a small volume (2 or 3 ml) of water.

Cold extraction: A total of 10gm of powdered sample was successively extracted with 50 ml distilled water and stirred magnetically (Magnetic stirrer- Snijders) in a container for 1½ hour at room temperature. The extract was filtered through filter paper and concentrated by a Rotary evaporator for just as long as was required to remove the solvent, and re-dissolved the residue in a small volume (2 or 3 ml) of water (Thirumalai et al., 2011).

Finally, the all extracts were collected in clean stoppered glass test tubes separately and used for phytochemical screening.

Same procedures were followed using ethanol and methanol instead of distilled water to prepare the cold and hot ethanol and methanol extracts.

Organoleptic Characters: The organoleptic characters of plant base products are evaluating the qualities of preparation by color, touch, fineness, taste, odor, etc. were noted through sense organs and it is providing the idea about the quality of different formulations without using chemical tests. Organoleptic characters of the root of W. somnifera crude powder, and it's

aqueous and alcoholic extracts were evaluated based on the method described by Siddiqui et al., 1995.

Preliminary Phytochemical Screening: The preliminary phytochemical screening of the various (cold & hot ethanol; methanol and water) extracts of root powder of W. somnifera were carried out according to standard laboratory procedures, to detect the presence of different secondary metabolites such as alkaloids, flavonoids, saponins, tannins, steroid glycosides, phenols, coumarins, reducing sugars, protein, anthraquinones, quinines, fat and fixed oil (Kokate et al. 1995; Farnsworth, 1996; Gupta et al. 2008; Prashant Tiwari et al. 2011 and Saxena et al., 2012).

Quantitative Estimations: Estimation of Total Alkaloid: Quantitatively, alkaloid was determined using the procedure forward by Harborne, 1973; as described by Edeoga et al., 2005 and Aliyu et al., 2008.

Briefly, five grams (5 g) of root powder was weighed into 250 ml beaker and 200 ml of 20% acetic acid was added and covered to stand for 4hr. This was filtered and the extract was concentrated using a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected by filtration through the accurately weighed filter paper. The residue is the alkaloid, which was dried at oven for 4 hours and weighed. Total alkaloid content was calculated as mg per g of air-dried material.

Estimation of Total Flavonoids: Flavonoids were determined using the procedure forward by Boham and Kocipaiabyazan (1994) as described by Edeoga et al., 2005 and Aliyu et al., 2008.

Briefly, 10 g of the root powder was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was then filtered using Whatman No:42 (125 mm) filter paper. The filtrate was later transferred into accurately weighed crucible and evaporated to dryness over a water bath and weighed to a constant weight. The weight is flavonoids. Total flavonoid content was calculated as mg per g of air-dried material.

Estimation of Total saponin (Determination of foaming index): Many medicinal plant materials contain saponins that can cause persistent foam when an aqueous decoction is shaken. The foaming ability of an aqueous decoction of plant materials and their extracts is measured in terms of a foaming index. Saponins were determined according to the method described by World Health Organization (WHO, 1998).

About 1g of the root powder weighed accurately and transferred to a 500ml conical flask containing 100ml of boiling water. Maintain at moderate boiling for 30minutes. Cooled and filtered into a 100ml volumetric flask and added sufficient water through the filter to dilute to volume. Poured the decoction into 10 stopper test-tubes (height 15cm, diameter 15mm) in series of successive portions of 1 ml, 2 ml, 3 ml, up to 10 ml and the volumes in each tube adjusted with water to 10 ml. Stoppered the tubes and shaken them in a lengthwise motion for 15 seconds, two shakes per second. After allowed the tubes to stand for 15 minutes and the height of the foam was measured by means of a graduated tape with millimetre scale.

Determination of volatile oil: Fresh roots of W. somnifera were washed to remove dirt, chopped into small pieces and ground in a blender. The material was subjected to hydro distillation using Clavenger-type glass apparatus for 4 hours separately. Then, observation done whether the volatile oil present or absent (WHO, 1998 & Hina Fazal et al. 2011).

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Table 1: Organoleptic characters of crude powder, aqueous and ethanolic extracts of the Root of W. somnifera

Name of the crude powder & extracts	Appearance	Colour	Taste	Odour
1. Crude powder	Powder	Whitish brown	Bitter	Characteristic
2. Aqueous extracts-				
Cold water extract	Liquid	Light brown	Bitter	Characteristic
Hot water extract	Liquid	Brown	Bitter	Characteristic
3. Alcoholic extracts-				
Cold ethanol extract	Liquid	Dark yellow	Bitter	Characteristic
Hot ethanol extract	Liquid	Orange	Bitter	Characteristic
Cold methanol extract	Liquid	Dark yellow	Bitter	Characteristic
Hot methanol extract	Liquid	Orange	Bitter	Characteristic

Statistical analysis: Statistical analysis of the results obtained in quantitative estimation was carried out by use of the Ms Excel 2007 statistical software and mean values along with standard deviation were recorded.

OBSERVATION AND RESULTS

The Organoleptic characters of aqueous and alcoholic extract of the root of W. somnifera, are tabulated as Table 1. The phytochemical active compounds of various extracts of root of W. somnifera were qualitatively analyzed and the results are presented in Table 2. The quantitative test for various functional groups is tabulated as Table 3.

Table 2: Phytocochemical Screening of various (cold & hot aqueous and alcohol) extracts of root of *W. somnifera*

Components	Various Extracts					
	Cold Ethanol	Hot Ethanol	Cold Methanol	Hot Methanol	Cold Aqueous	Hot Aqueous
Phenolic compound	+++	+++	+++	+++	+++	+++
Flavonoids-						
Shinoda test	+++	+++	+++	+++	+	+
Coumarins	+++	+++	+++	+++	+++	+++
Quinones	0	0	0	0	0	0
Anthraquinones	0	0	0	0	0	0
Tannins-						
Ferric chloride test	+++	+++	+++	+++	+++	+++
Saponins-						
Foam test	++	++	++	++	+	+
Protein-						
Xanthoproteic Test	++	++	++	++	++	++
Steroid-glycosides-						
Libermann Burchard's test	+++	+++	+++	+++	++	++
Alkaloids						
Mayer's Test	+++	+++	+++	+++	+++	+++
Dragendroff's Test	+++	+++	+++	+++	+++	+++
Reducing sugars-						
Fehling's test	+++	+++	+++	+++	+++	+++
Fixed oil and Fats	0	0	0	0	++	++

+++ = appreciable amount, ++ = average amount, + = trace amount, **0** = absent

Table 3: Total alkaloids, flavonoid and saponin contents in root powder of root of *W. somnifera*

Name of the plant material	Total alkaloids	Total flavonoids	Total saponins (Foaming Index)
Root powder of *W. somnifera*	0.81 ± 0.01	14.43 ± 0.40	FI < 100

Values are expressed as mean% ± S.D., n=3

DISCUSSION

Secondary metabolites were found in good proportion in alcoholic and aqueous extracts when compared. These secondary metabolites may be responsible for various pharmacological effects of ethanolic, methanolic and aqueous extracts of preparations. Such preliminary phytochemical screening was helpful in prediction of nature of drugs and also useful for the detection of different of different constituents present in different polarity solvent.

So it could be helpful to extract out particular constituents by solvent (Shwetajain, et al., 2011).

The secondary metabolites such as alkaloids, flavonoids, lignins, terpenoids, steroids, glycosides, coumarins and phenols in plant materials produce the curative effect when they are used in the traditional medical practice (Sane et al., 1997).

As seen in Table 1, both the aqueous and alcoholic extracts of Root powder of W. somnifera had similar organoleptic properties except for the colour of the each extracts. In analysis of Tannin compounds brownish green colour developed to indicate the presence of Tannin. Similarly based on the presence or absence of colour change indicate positive and negative results. In these screening process alkaloids, saponins, flavonoids, steroids, tannins, coumarins, phenols, proteins, reducing sugars, fixed oil and fats gave positive results and anthraquinones and quinones gave negative results for cold and hot ethanol, methanol and water extracts of root powder of W. somnifera. Cold and hot water extracts showed the presence of fats and fixed oil. Higher flavonoids, steroidal glycosides and saponins content were found in the cold and hot ethanol and methanol extracts than in the cold and hot water extracts of root of W. somnifera.

Earlier studies for phytochemical screening that presence of alkaloids, flavonoids, steroids, tannins, terpenoids, saponins and sugars in alcoholic root extract (Brijendra et al., 2010); presence of alkaloids, flavonoids, saponins, steroids, and tannins in crude extract of W. somnifera (Khan et al., 2010; Mukesh & Smita 2010) presence of alkaloids, flavonoids, tannins, saponins, glycosides, phenols, protein, phytosterol, triterpenoids and reducing sugars in ethanolic root extract (Bimlesh Kumar et al., 2011) and presence of terpenoids, alkaloids, saponins, carbohydrates, glycosides, flavonoids, tannins and steroids in Hydromethanolic root extract (Nasreen & Radha 2011)

As seen in Table 3, the total alkaloid (20% acetic acid extract) and flavonoid (80% of aqueous methanol extract) contents were found to be 0.81 ± 0.01% and 14.43 ± 0.40% respectively and total saponin (hot water extract) content was (Foaming Index) FI < 100. The considerable amount of volatile oil was not determined in fresh root of W. somnifera.

Previous study stated that, the total alkaloid content was found to be 0.9818mg/100g of root powder of W. somnifera (Nasreen & Radha 2011).

CONCLUSION

The present study has revealed the presence of many secondary metabolites in the various extracts of root of W. somnifera powdered preparation. It has the further confirmed that these plant extracts could be used for the treatment of various ailments. The findings are consistent with the presence of biologically active constituents in the polar extracts of W. somnifera and may provide helpful in authentication and identification of this plant.

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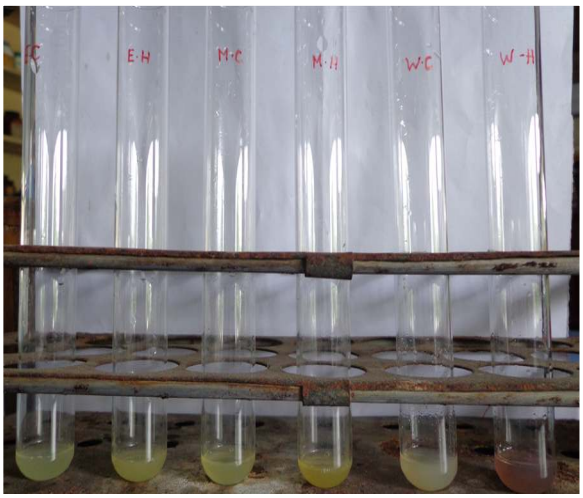
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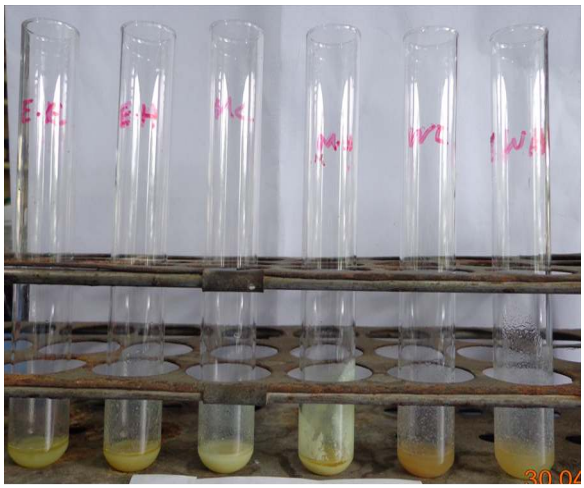
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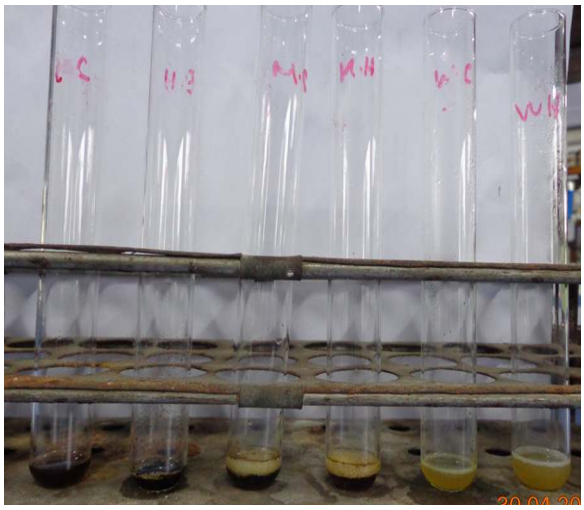
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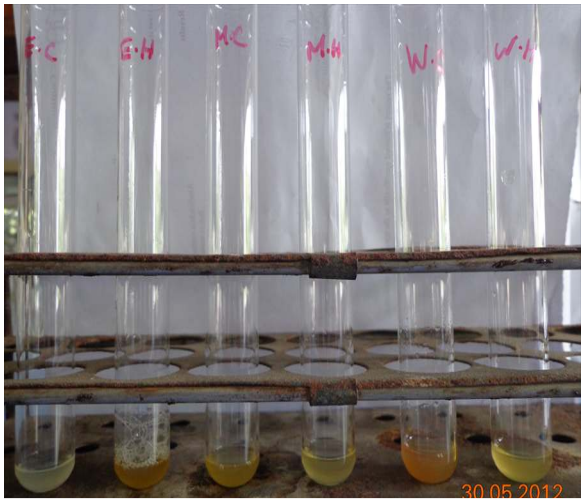
Alkaloids in *Withania*



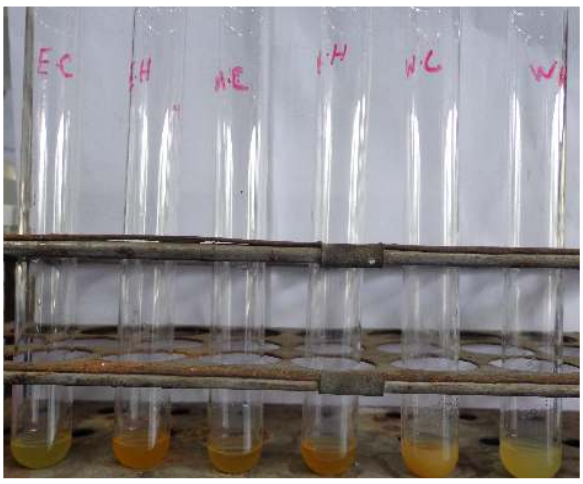
Coumarins in *W. somnifera*



Flavonoids in *W. somnifera*



Anthraquinones in *W.*



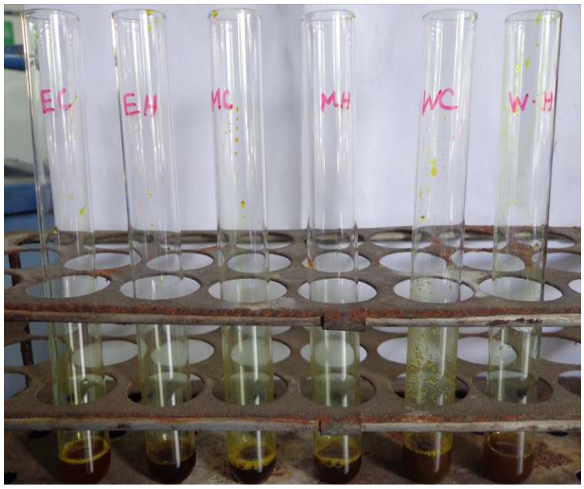
Proteins in *W. somnifera*



Phenolics in *W. somnifera*



Saponins in *Withania*



Tannins in *W. somnifera*