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INVITRO ANTIOXIDANT ACTIVITY OF ROOT EXTRACT OF CURCUMA LONGA (CL) AND WITHANIA SOMNIFERA (WS)

Pharmaceutical Science

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ABSTRACT

The reactive oxygen species can be deleterious to various vital cellular components such as lipids, proteins and DNA which can, in the due course of time lead to diseases like diabetes, neurodegenerative and cardiovascular diseases, arthritis etc. Much light has been thrown on the research to obtain the natural sources of antioxidants which can be consumed through diet so that they protect the biological systems from oxidative damage. Hence anti-oxidant activity of root extracts (aqueous, ethanolic, water-ethanolic) of easily available plants such as Curcuma longa (Turmeric) and Withania somnifera (Ashwagandha) which are known to possess anti-oxidant and immunomodulatory properties were studied using DPPH and Nitric Oxide scavenging assays. Ethanolic extract of Curcuma longa (69.42%) and water-ethanolic extract of Withania somnifera (68.37%) showed the highest anti-oxidant activity. The standard used was ascorbic acid. Both the drugs prove to be promising candidates from which bioactive elements could be developed.

KEYWORDS

Antioxidants, Curcuma longa, Withania somnifera, Scavenging assays

INTRODUCTION

Various forms of activated oxygen, termed as reactive oxygen species (ROS) including free radicals and non-free radicles are produced by various biological processes or exogenous reactions. Free radicals are named so as they possess one or more free electrons. ROS have been a double-edged sword as they are found to be beneficial in signal transduction as well as can be deleterious to various vital cellular components such as lipids, proteins and DNA which can, in the due course of time lead to diseases like diabetes, cardiovascular diseases, neurodegenerative and gastrointestinal diseases, aging, atherosclerosis, arthritis and other degenerative diseases in humans (1,2).

Anti-oxidants exhibit their property via a number of mechanisms such as blocking the chain initiation, hydrogen abstraction, peroxide decomposition, binding of transitional metal ion catalysts and scavenging of free radical. Much light has been thrown on the research to obtain the natural sources of antioxidants which can be consumed through diet so that they protect the biological systems from oxidative damage, delay the progression of many chronic diseases and also are associated with fewer side effects (1,2).

Curcuma longa, commonly known as turmeric belongs to the family, *Zingiberaceae.* Curcumin which is the biologically active constituent has shown to possess anti-inflammatory, antioxidant, anticarcinogenic and antimutagenic properties (3).

Withania somnifera, commonly called as Ashwagandha belongs to the family Solanaceae. The rhizomes of the plant are of main therapeutic values. The root extract of W. somnifera has shown immune-modulatory and anti-tumor activity (4,5). The hydroalcoholic root extract has exhibited chemoprotective properties which may be due to the antioxidant or the free-radical quenching ability of the extract constituents (6).

This study was conducted to determine the free-radical scavenging activity of *Curcuma longa* and *Withania somnifera* and also to compare the anti-oxidant activity of different extracts (aqueous, ethanolic and water-ethanolic) of the roots of the two plants.

MATERIALS AND METHODS

Chemicals And Reagents

1,1-diphenyl-2-picrylhydrazyl (DPPH), ethanol, distilled water, sodium nitric oxide, glacial acetic acid, N-(1-Naphthyl) ethylenediamine dihydrochloride and sulfanilic acid.

Instrumentation

Electric water bath, electric grinder, vaccum filter, ultra-sonicator, UV chamber and incubator.

Plant Collection

The roots of Curcuma longa and Withania somnifera was collected from an organic store in Bangalore, Karnataka. The roots were identified and authenticated by Dr.V. Rama Rao, Research Officer (Botany), Central Ayurvedic Research Institute, Bangalore, India. The rhizomes were dried in shade and finely powdered using and electric grinder.

Extraction

Extraction was carried out conventionally using the reflux method. 20 g of powdered plant material was mixed with 200 ml of distilled water in a round bottom flask and refluxed for about 5 h at 100 C. 20 g powder plant material was mixed with 200 ml of ethanol. Similarly, 20 g of powder was mixed with ethanol and water–ethanol (9:1) separately in round bottom flasks and refluxed for 5 h. Liquid extracts obtained were separated from the solid residue by Whatman filter paper, followed by which, the filtrate was evaporated by using electric water bath in a China dish at 70˚C (7).

DPPH Scavenging Activity

Radical Scavenging activity of Curcuma longa (CL) and Withania somnifera (WS) was measured according to the Blois method (8). Approximately, 1ml of varying concentrations of the extract (62.5-500µg/ml of ethanol, water-ethanol and water) was added to 1ml of DPPH solution (0.2 mM in ethanol) which acts as a source of free oxidative radicle. This preparation was incubated at room temperature for 30 minutes. Drop in the solution absorbance as a result of proton donating activity of CL and WS was measured at 490nm using microtiter plate reader (ELISA) against the corresponding test and standard blank. Control samples were prepared similarly as that of the test samples with an equal volume of buffer without the extracts. L-Ascorbic acid served as the positive control. The percentage DPPH radical scavenging property was determined by using the formula:
DPPH radical scavenging activity (%) = $[(A_0-A_1)/A \times 100]$ where, A_0 indicates the absorbance of control and A_1 indicates the absorbance of standard sample.

Nitric Oxide-scavenging Activity

The nitric oxide scavenging activity was determined according to the Sreejayan & Rao method (9). Varying concentrations of the extract was mixed with Sodium nitroprusside (10 mM) in phosphate-buffered saline and incubated at 25^{0}C for 150 min. Post incubation, Griess reagent (0.5 ml), which constitutes 1% sulphanilamide, 2.5% phosphoric acid (H_3PO_4) and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, was added to the mixture incubation. Control samples were prepared similarly as that of the test samples with an equal volume of buffer without the extracts. T he absorbance of pink-colored chromophore was read at 540nm against the corresponding test and standard blank. L-Ascorbic acid served as the positive control.
NO scavenging activity (%) = $[(A_0-A_1)/A \times 100]$ where, A_0 indicates the absorbance of control and A_1 indicates the absorbance of standard sample.

RESULTS

Ethanolic extract of *Curcuma longa* showed high DPPH and Nitric oxide scavenging activity, compared to the aqueous and water-ethanolic extract, using ascorbic acid as the standard. (Table 1)

Table 1: Free Radical Scavenging Activity Of Aqueous, Ethanol And Aqueous-ethanolic Root Extracts Of Curcuma Longa Linn

Solvent	DPPH (%)	NOSA (%)
Aqueous	61.59 ± 1.28	39.08 ± 1.31
Ethanol	69.42 ± 1.82	49.84 ± 1.18
Aqueous-Ethanol	63.56 ± 2.20	43.55 ± 2.29
Ascorbic Acid (STANDARD)	89.14 ± 0.313	91.52 ± 1.28

Results are expressed as mean ± SEM (n=3).

Water - ethanolic extract of *Withania somnifera* showed high DPPH and Nitric oxide scavenging activity, compared to the aqueous and ethanolic extract, using ascorbic acid as the standard. (Table 2)

Table 2: Free Radical Scavenging Activity Of Aqueous, Ethanol And Aqueous-ethanolic Root Extracts Of Withania Somnifera

Solvent	DPPH (%)	NOSA (%)
Aqueous	62.22 ± 7.11	39.74 ± 5.25
Ethanol	65.41 ± 8.50	43.32 ± 6.01
Aqueous-Ethanol	68.37 ± 7.30	48.32 ± 6.01
Ascorbic Acid (STANDARD)	89.14 ± 0.313	91.52 ± 1.28

Results are expressed as mean ± SEM (n=3).

DISCUSSION

DPPH method has its principle based on the reduction of alcoholic DPPH solution in the presence of antioxidant which is hydrogen donating (1). From the above results we find that the ethanolic extract of Curcuma Longa and water ethanolic extract of Withania somnifera were able to neutralize the DPPH free radicals by 69.42% and 68.37% respectively.

Nitric oxide upon reaction with oxygen molecule produce stable nitrate and nitrite oxidative species which is estimated by Griess reagent. The decrease in the amount of nitrous acid in the presence of scavenging test compound is measured at 540nm (1). The nitric-oxide scavenging activity results suggest that that the ethanolic extract of Curcuma Longa and water ethanolic extract of Withania somnifera were able to neutralize the NO free radicals by 49.84% and 48.32% respectively.

CONCLUSION

The current study is a preliminary study as it is based on crude root extract and hence demands further advanced studies to draw appropriate confirmatory conclusions. The study findings suggest that Curcuma longa and Withania somnifera can be promising plants for further investigation of its anti-oxidant and immunomodulatory properties, in which case it can be carried out on extracts other than that of root such as the rhizomes or leaf to determine the highest anti-oxidant activity. Both the drugs prove to be promising candidates from which bioactive elements could be developed.

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REFERENCES

1. Sharma, S. and Singh, A., 2012. In Vitro Antioxidant and Free Radical Scavenging Activity of Nardostachys jatamansi DC. Journal of Acupuncture and Meridian Studies, 5(3), pp.112-118.
2. Sharma, U. and Kumar, A., 2011. In vitro antioxidant activity of Rubus ellipticus fruits. Journal of Advanced Pharmaceutical Technology & Research, 2(1), p.47.
3. Motterlini, R., Foresti, R., Bassi, R. and Green, C., 2000. Curcumin, an antioxidant and anti-inflammatory agent, induces heme oxygenase-1 and protects endothelial cells against oxidative stress. Free Radical Biology and Medicine, 28(8), pp.1303-1312.
4. Davis, L. and Kuttan, G., 2000. Immunomodulatory activity of Withania somnifera. Journal of Ethnopharmacology, 71(1-2), pp.193-200.
5. Shahriar, M., 2013. InVitro Antioxidant and Free Radical Scavenging Activity of Withania Somnifera Root. IOSR Journal of Pharmacy (IOSRPHR), 3(2), pp.38-47.
6. Prakash, J., Gupta, S. and Dinda, A., 2002. Withania somnifera Root Extract Prevents DMBA-Induced Squamous Cell Carcinoma of Skin in Swiss Albino Mice. Nutrition and Cancer, 42(1), pp.91-97.
7. Dhanani, T., Shah, S., Gajbhiye, N. and Kumar, S., 2017. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of Withania somnifera. Arabian Journal of Chemistry, 10, pp.S1193-S1199.
8. BLOIS, M., 1958. Antioxidant Determinations by the Use of a Stable Free Radical. Nature, 181(4617), pp.1199-1200.
9. Sreejayan N, Rao MN. Free radical scavenging activity of curcuminoids. Arzneimittelforschung. 1996 Feb;46(2):169-71.
10. Mohammed, A., Usman, M., Wudil, A., Alhassan, A., Abubakar, S. and Lat, N., 2020. In vitro and in vivo Antioxidant Properties of Extracts from the Root of Curcuma longa Linn. European Journal of Medicinal Plants, pp.6-12
11. Sumathi S, Padma PR, Gathampari S, Vidhya S. Free radical scavenging activity of different parts of withania somnifera. Anc Sci Life. 2007 Jan;26(3):30-4.