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Research Article

Antioxidant Activity of Formulation Containing Mixture of Herbs

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ABSTRACT

Reactive oxygen species (ROS) are thought to underline the process of ageing and the pathogenicity of various diseases, such as neurodegenerative disorders and cancer. The use of traditional medicine is widespread and plants still present a large source of natural antioxidants that might serve as leads for the development of novel drugs. The purpose of our study is to investigate the antioxidant activity of the formulations containing roots of *Withania somnifera* family *Solanaceae*, leaves of *Ocimum sanctum* family *Lamiaceae*, and rhizome of *Curcuma longa* family *Zingiberaceae*.

Keywords: 2, 2-Diphenyl-1-Picryl Hydrazyl (DPPH), Free radical, ashwagandha.

INTRODUCTION

Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and our metabolism. They are continuously produced by the body's normal use of oxygen such as respiration and some cell mediated immune functions. The oxygen consumption inherent in cell growth leads to the generation of a series of reactive oxygen species (ROS). ROS, which include free radicals such as superoxide anion radicals ($O_2^{\cdot}$), hydroxyl radicals ($OH^{\cdot}$) and non-free radical species such as hydrogen peroxide (H_2O_2) and singlet oxygen (O_2), are various forms of activated oxygen. Accordingly, ROS is a collective term that includes both oxygen radicals and certain non-radicals that are oxidizing agents and / or easily converted into radicals (HOCl, HOBr, O_2, $O_2^{\cdot}$, H_2O_2). All oxygen radicals are ROS, but not all ROS are oxygen radicals. Similarly, reactive nitrogen species (RNS) are mainly nitric oxide ($NO^{\cdot}$), peroxynirite ($ONOO^{\cdot}$) and nitrogen dioxide (NO_2). ROS can be formed in living organisms by both endogenous and exogenous sources. Endogenous sources of free radicals include normal aerobic respiration, peroxisomes and stimulation of polymorphonuclear leukocytes and macrophages. The exogenous sources include ionizing radiation, tobacco smoke, pollutants, pesticides and organic solvents (Umamaheswari and Chatterjee, 2008).

The interaction of ROS species with molecules of a lipid nature produces new radicals: hydroperoxides and different peroxides (Kumran and Kaurunakaran, 2006). ROS are continuously produced during normal physiological events and can easily initiate the peroxidation of membrane lipids, leading to the accumulation of lipid peroxides. ROS is capable of damaging crucial biomolecules such as nucleic acids, lipids, proteins and carbohydrates (Rajeshwar and Senthil, 2005).

The harmful action of free radicals can, however, is blocked by antioxidant substances. Antioxidant compounds may function as free radical scavengers, complexers of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation (Huang and Ou, 2005). They can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells and prevent damage to lipids, proteins, enzymes, carbohydrates and DNA (Lee *et al*., 2004).

Antioxidants can be classified into two major classes i.e. enzymatic and non-enzymatic. The enzymatic antioxidants are produced endogenously and include super oxide dismutase, catalase and glutathione peroxidase. The non-enzymatic antioxidants include tocopherol, carotenoids, ascorbic acid, phenolic compounds, vitamin E, flavonoids and tannins which are obtained from natural plant sources (Grice, 1986). A wide range of antioxidants from natural and synthetic origin has been proposed for use in the treatment of various human diseases. There are some synthetic antioxidants compounds such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary butyl hydroquinone which are commonly used in food processes (Formica and Regelson,

1995). However, it has been suggested that these compounds have shown toxic effects such as liver damage and mutagenesis. Flavonoids and other phenolic compounds of plant origin have been reported as scavengers of free radicals. Hence, the search for natural antioxidants source is gaining importance (OECD Guideline 423., 2001).

PLANT MATERIAL

Roots of Ashwagandha were collected in August 2014 from local market of Mumbai.

Leaves of Tulsi were collected in August 2014 from botanical garden of Dr. Bhanuben Nanavati College of Pharmacy, Mumbai.

All plants were taxonomically identified and authenticated by Dr. Bindu Gopalkrishanan, Botany department at Mithibai College, Vile Parle, Mumbai.

The sample specimen was preserved in our laboratory for future reference.

Haldi extract was collected in August 2014 from Sami labs, Bangalore.

PLANT PROCESSING

The roots of Ashwagandha were dried under shade; then pulverized by a mechanical grinder, passed through a 40- mesh sieve, and stored in a well closed container for future use.

The leaves with stalks of Tulsi were dried under shade; then pulverized by a mechanical grinder, passed through a 40- mesh sieve, and stored in a well closed container for future use.

PREPARATION OF EXTRACTS

100 g of the dried and pulverized roots of Ashwagandha was extracted with hydroalcoholic solvent (distilled water: ethanol) (1:1) in a Soxhlet apparatus. The solvent was removed from extract under vacuum rotary dryer and a semi solid mass (18.46 % w/w in respect of dry material) was obtained. Finally, the extract was stored in a vacuum desiccator. The dried extract thus obtained was used for the assessment of phytochemical screening and antioxidant activity through various *in vitro* assays (Jain *et al*., 2004).

100 g of the dried and pulverized leaf of Tulsi was extracted with hydroalcoholic solvent (distilled water: ethanol) (1:1) in a Soxhlet apparatus. The solvent was removed from extract under vacuum rotary dryer and a semi solid mass (19.26 % w/w in respect of dry material) was obtained. Finally, the extract was stored in a vacuum desiccator. The dried extract thus obtained was used for the assessment of phytochemical screening and antioxidant activity through various *in vitro* assays (Ramesh and Satakopan, 2010).

PHYTOCHEMICAL SCREENING

- The hydroalcoholic extract of the roots of Ashwagandha was subjected to preliminary phytochemical screening and it contains steroidal lactones like withanolide, alkaloids like withanines, somniferine, acyl steryl glucosides, monohtdrate alcohol and fatty acids etc (Kokate *et al*., 2007).
- The hydroalcoholic extract of the rhizome of Haldi was subjected to preliminary phytochemical screening and it contains terpenoids curcumin, polyphenols,volatile oil, resins, starch grains etc (Kokate *et al*., 2007).
- The hydroalcoholic extract of the leaves of Tulsi was subjected to preliminary phytochemical screening and it contains terpenoids, volatile oils like eugenol, linalool, alkaloids, glycosides and saponins etc (Gautam and Goel, 2014).

Preliminary phytochemical screening was performed to confirm that the extract contains constituents that it claims to possess. The extract was subjected to preliminary phytochemical evaluation using qualitative chemical tests for detecting the presence of the phytoconstituents such as alkaloids, glycosides, tannins, flavonoids, steroids, carbohydrates, proteins and amino acids etc (Kokate *et al*., 2007)

PREPARATION OF FORMULATION

In order to perform psychopharmacological evaluation of formulations for anti-parkinson's activity, all three plant extracts were mixed (i.e. Ashwagandha, Tulsi and Haldi) in three different concentrations keeping final dose constant i.e. 300mg/kg.

Formulation I	Ashwagandha 100mg/kg + Tulsi 100mg/kg + Haldi 100mg/kg
Formulation II	Ashwagandha 150mg/kg + Tulsi 100mg/kg + Haldi 50mg/kg
Formulation III	Ashwagandha 50mg/kg + Tulsi 150mg/kg + Haldi 100mg/kg

DPPH RADICAL SCAVENGING ACTIVITY

Method: The free radical scavenging activity of extract was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH (2, 2-diphenyl-1-picrylhydrazyl) (Rajeshwar and Senthil, 2005, Formica and Regelson, 1995).

Principle: DPPH (2, 2-diphenyl-1- picrylhydrazyl) is a stable free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. Due to its odd electron, the ethanolic solution of DPPH shows a strong absorption band at 517 nm. DPPH radicals react with suitable reducing agents and then electrons become paired off and the solution loses colour stiochometrically with the number of electrons taken up. Such reactivity has been widely used to test the ability of plant extracts to act as free radical scavenger. Reduction of DPPH radicals can be observed by the decrease in absorbance at 517 nm.

Procedure: 0.1 mM solution of DPPH in ethanol was prepared and 1.0 ml of this solution was added to 3.0 ml of dilutions of extract solution in water at different concentrations (10-100 µg/ ml). Thirty minutes later, the absorbance was measured at 517 nm. Ascorbic acid was used as standard. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

Calculation: Assay was done in triplicates. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{Scavenging Effect (\%)} = (A_{control} - A_{test} / A_{control}) \times 100$$

Where A_{cont} is the absorbance of the control reaction and $A_{test/std}$ is the absorbance of the extracts/ standards.

RESULT

DPPH FREE RADICAL SCAVENGING ACTIVITY

Table 1: DPPH free radical scavenging activity of hydroalcoholic extract and ascorbic acid

Concentration (µg/ ml)	Scavenging effect % (plant extract)	Scavenging effect % (ascorbic acid)
10	24.50 ± 0.234	34.33 ± 0.145
20	36.55 ± 0.178	45.86 ± 0.178
40	54.65 ± 0.156	63.78 ± 0.156
60	66.36 ± 0.123	74.34 ± 0.123
80	75.33 ± 0.149	82.79 ± 0.149
100	81.36 ± 0.180	89.17 ± 0.180

Values are expressed as a mean ± SEM of 3 observations

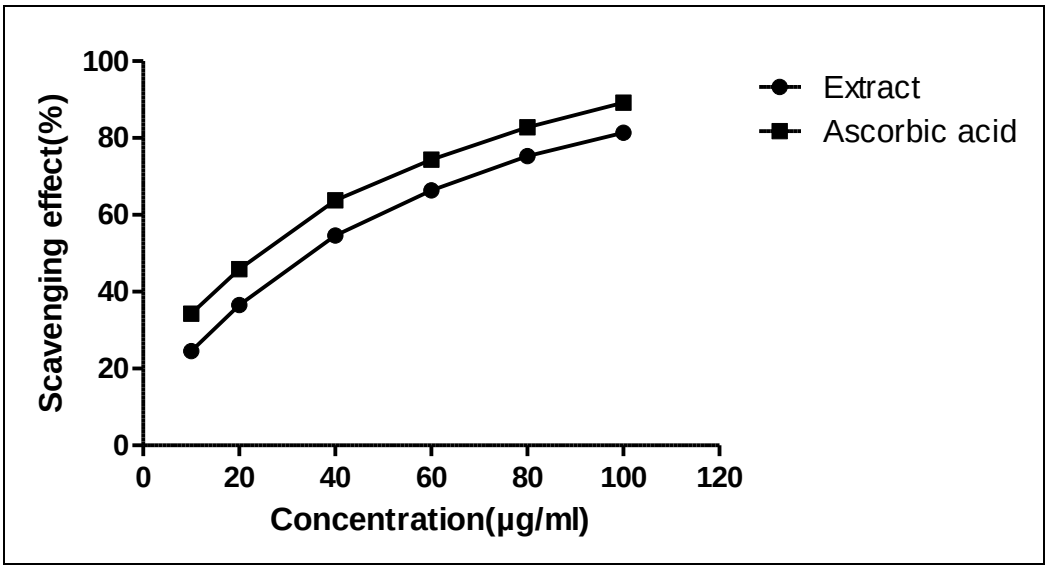

Fig. 1: Comparison of DPPH free radical scavenging activity of plant extract and ascorbic acid

The scavenging percentage on the DPPH radical was 51.48 % for mixture of hydroalcoholic extract of *Withania somnifera, Ocimum sanctum* and *Curcuma longa* at the dose level of 40 µg and 64.26 % for ascorbic acid at the same dose i.e. 40 µg. The half-effective dose (ED_{50}) for mixture of hydroalcoholic extract of *Withania somnifera, Ocimum sanctum* and *Curcuma longa* was found to be 38.50 µg and for ascorbic acid was 19.50µg.

CONCLUSION

From the above results and data conclude that from formulations I, II, III, formulation II shows best antioxidant effect. Because it contains higher amount of ashwagandha which is a nerve tonic, then tulsi similarly work like mucuna which contains naturally occurring DA and haldi helps to reduce the alpha synuclein aggregation.

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