In vitro Free Radical Scavenging Study on Various Extracts of Radix *Operculina turpethum* (L.) Silva Manso (Black and White Varieties) to Combat Oxidative Stress

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Abstract

Oxidative stress produces detrimental effects on body leading to multiple diseases including cancer, neurodegenerative, and cardiovascular diseases. Nature has gifted human kind a variety of medicinal plants having antioxidant potential. *Operculina terpethum* is one of the important plants of traditional system of medicines. Its black and white variety was selected for the study of radical scavenging activity. Antioxidant activity of both black and white varieties of *O. turpethum* was studied by calculating free radical scavenging activity using DPPH assay. Roots of both varieties were extracted with Ethanol that was further fractionated with butanol and ethyl acetate. All of these extracts were tested using DPPH assay taking Ascorbic acid as control. Absorbance was recorded at 517 nm. Results of study proved that both varieties of *operculina turpethum* possess antioxidant potential in dose dependent manner in all extract of both varities. More over IC_{50} also showed good inhibition in butanol extract. Both varieties of *O.turpethum* showed excellent anti-oxidant potential thus can be applied to treat and prevent diseases caused by excessive oxidative stress.

Keywords

Roots extract, antioxidant, DPPH, percent inhibition, Indian jalap, IC_{50}.

1. INTRODUCTION

Since ages *Operculina turpethum* L. Silva Manso (family *convolvulaceae*) is a notable medicinal herb widely used in traditional system of medicines especially in Unani and Ayurvedic. It is commonly known as Indian Jalap or turpeth and distributed in Pakistan, India, China, Asia and Australia (Ahmad *et al.,* 2017 ; Austin, 1982; Khare, 2007). *Operculina turpethum* L. is available in two varieties black and white exhibiting a wide range of medicinal actions like expectorant, bronchodilator, antioxidant, anti inflammatory, analgesic, anti microbial and laxative.

Based on these actions this plant has numerous therapeutic uses to treat different gastrointestinal diseases, cough, asthma, spleenomegaly, anemia, high lipid profile and obesity (Shareef *et al.,* 2014; Shareef *et al.,* 2010; Kumar *et al.,* 2006). Phytochemical screening of *O. turpethum* L. has been explored by researchers confirming the presence of valuable constituents that render this plant not only a significant therapeutic agent in traditional system of medicines rather a suitable candidate for further pharmaceutical research. Some major reported compounds are phytosterol, sitosterols, terpenes, flavonoids, phenol, cardiac glycoside and volatile oil etc (Ahmad *et al.,* 2017; Anbuselvam *et al.,* 2007; Shah *et al.,* 1972; Wenbing *et al.* 2011).

Oxidative stress produces detrimental effects in the body and is a major cause of different ailments. Researchers have proven that increased oxidative burden not only initiate cancerous cell growth but it contribute in invigorating different diseases like diabetes, cardiovascular diseases, neurological problems like Alzheimer, Parkinson disease, delayed sexual maturation etc. Therefore, plants having antioxidant activity can promote cellular longevity thus play important role in decreasing the disease burden caused by oxidative degeneration (Pizzino *et al.*2017; Rajendran*, et al.*2014; Kumar and Pandey, 2015; Valko *et al.,* 2007; Sharma and Singh, 2012).

Therefore, in this research study of both black and white varieties of medicinal plant *Operculinaturpethum* were selected to explore their antioxidant profile and prove this plant as a suitable candidate to treat different diseases caused by detrimental effects of oxidative load on the body.

2. MATERIALS AND METHODS

2.1. Collection and Identification of Plant Material

Roots of two varieties of *Operculina turpethum* i.e. black and white were selected to explore antioxidant potential. Samples of plants were collected from local herbal market of Karachi city and identified and authenticated by Prof. Dr. Ghazala H Rizwani.

2.2. Extract Preparation

The collected plant material of both varieties of *O. turpethum* was garbled, washed and shade dried. Extraction of each plant variety was performed by soaking in Ethanol for 15 days at room temperature. It was then filtered through whatman filter paper No 1, followed by solvent evaporation under reduced pressure using rotary evaporator. The dried Ethanolic extract thus obtained was fractionated with Butanol and Ethyl acetate in the order of polarity. The solvents of theses fractions were evaporated using rotary evaporator obtaining the extract of butanol and ethyl acetate.

2.3. Chemicals and Reagents

All chemicals and reagents used were Analytical grade and purchased from Sigma Aldrich. Ethanol, Butanol, ethyl acetate, and 2, 2 –diphenyl-1-picrylhydrazyl (DPPH) were used.

2.4. Antioxidant Activity using DPPH Free Radical Scavenging Assay

Free radical scavenging activity of ethanolic, butanol, and ethyl acetate extracts of *O. turpethum*

(black and white varieties) were analyzed by DPPH assay following the procedure of *Huma - et al*, 2019.

2.4.1. Sample preparation and Incubation

In this regard various concentrations of each extract (20, 40, and 60 mg/ml) were prepared and tested for antioxidant potential. Ascorbic acid (0.6 mg/ml) was taken as standard and DPPH solution was used as blank.

All samples (test samples, standard, and blank) were freshly prepared and incubated for 30 minutes prior to analysis.

2.4.2. Absorrbance using UV Spectrophotometer

After incubation period, absorbance of all extracts of *O.turpethum* (black and white verities) at various concentrations as well as Ascorbic acid (standard) were recorded using spectrophotometer at 517 nm. All observations were recorded as triplicate. These recorded absorbencies of each extract and standard were then used to measure % inhibition using following formula;

% inhibition = Absorbance of control-Absorbance of Sample / Absorbance of control *100

2.4.3. IC_{50} value

IC_{50} values were calculated for each extract after assessment of % inhibition by plotting graph between Extract Concentration verse percent inhibitions.

3. RESULTS AND DISCUSSION

Oxidative stress is found as a progressive cause of not only cancer but also different chronic diseases like diabetes, cardiac and neurodegenerative diseases especially age related complications.

Human body is equipped with different natural antioxidant like glutathione, ascorbic acid (vitamin C), tocopherol (vitamin E) , superoxide dismutase etc that provide defensive mechanism against peroxides or free radicals. Inhibition of free radicals ultimately prevents progression of diseases. Body's natural antioxidant potential can be strengthen by utilizing medicinal plants having antioxidant phyto chemicals (Anbusalvum *et al*., 2007; Shareef *et al.,* 2014).

In this research study two varieties of *O.turpethum* black and white have been assayed for free radical scavenging activity by DPPH method. The plant material was extracted with ethanol, followed by fractionation with butanol and ethyl acetate. Antioxidant profile of each extract of *O. turpethum* at different concentrations (20, 40, and 60 mg/ml) was generated via calculating its respective % free radical scavenging activity (% inhibitions value).

Results proved that roots of both varieties of this plant possess antioxidant potential in dose dependent manner. Figure 1 represents comparison of antioxidant potential among various root extracts of White variety of Indian jalap at doses of 20, 40, and 60 mg/l keeping Ascorbic acid as Standard. It can be shown that maximum inhibitory effect was detected in butanol fraction (91% at dose of 60 mg/ml).

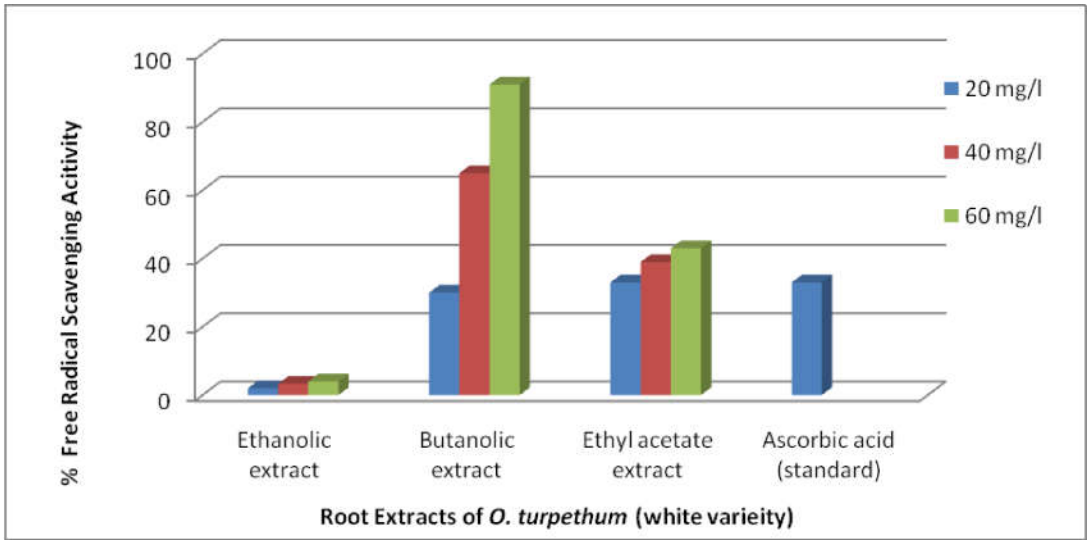

Figure 1: Comparison of Antioxidant potential among various Root extracts of *white variety of Operculina turpthum*

Figure 2 gives a graphic representation of antioxidant potential of different root extracts of black variety of Indian Jalap at doses of 20, 40, and 60 mg/l keeping Ascorbic acid as standard. In case of black variety of *O. turpethum* maximum % radical scavenging activity was recorded as 80% at the dose of 60 mg/ml (Figure 2).

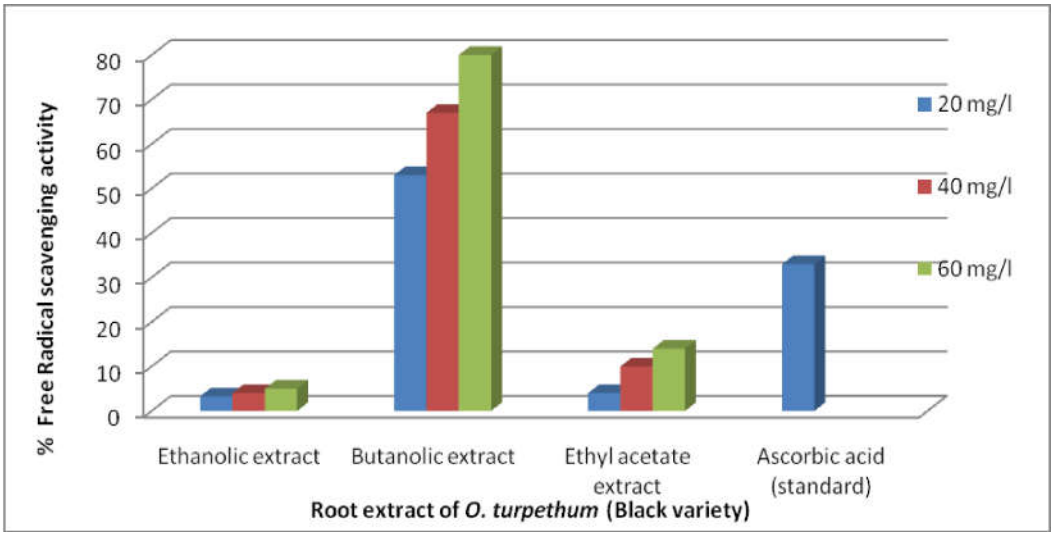

Figure 2: Comparison of Antioxidant potential among various root extracts of black variety of *Operculina turpethum*

After exploring the antioxidant potential, next step was to calculate the IC $_{50}$ value of each extract of *O.turpethum* (black and white varieties). Table 1 Represents the IC_{50} values.

Table 1: IC_{50} Values of Different Root Extracts of White and Black Varieties of *O. turpethum*

Root Extracts of *O. turpethum*	IC_{50} Values	
	White Variety	Black Variety
Ethanol Extract	12	45
Butanol Extract	32	58
Ethyl acetate Extract	111	4.6

Our results are in agreement with the findings of other researchers which shows the potential antioxidant capacity of different parts of this plantlike Anbusalvum *et al*., 2007; Sharma and Singh 2012 and Ezeja *et al*., 2015 whereas, our research particularly shows significant potential on roots of both verities.

4. CONCLUSION

Both varieties of *Operculina turpethum* (black and white) have been proved as free radial scavengers showing strong antioxidant activity. This medicinal action render *O. turpethum* a potential candidate to be used in pharmacy for the prevention and treatment of diseases caused by increased oxidative stress like Alzheimer's, Parkinson's diseases, diabetes, cancers etc.

Authors' Contributions

HS: Concept Study design, data collection and interpretation, BH: Data analysis and interpretation, Drafting of manuscript. HZ: Experimental FI: manuscript writing and Critical review SI: data analysis and Review manuscript, GHR: supervised the research.

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