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Research Article

Journal of Integral Sciences

Antioxidant activity of *Grewia villosa*

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Received: 7 Oct 2018; Revised: 22 Oct 2018; Accepted: 27 Oct 2018

Abstract

Oxidative stress is one of the main cause in improvement of side effects and infectious conditions in body. So, there is a need to identify more antioxidants to reduce oxidants and their easy availability. Natural resources like medicinal plants are easily available sources as antioxidants because of their edibility and less side effects. In this point of view, the present work was carried out to identify antioxidant activity of *Grewia villosa* root part and preliminary phytochemical analysis. The antioxidant activity was carried out on superoxide, hydroxyl and DPPH free radical for ethyl acetate and hydro-alcoholic extracts of *Grewia villosa* root. The extracts of *Grewia villosa* showed presence and absence of different phytochemical constituents and showed concentration dependent percentage reduction on tested free radicals. The hydro-alcoholic extract showed more activity compared to ethyl acetate extract and the extract showed more activity on DPPH free radical compared to other free radicals. The current research confirms antioxidant activity of *Grewia villosa* root extracts and further research is worth full to identify responsible bioactive compounds from these extracts on reduction of oxidative stress and other harmful diseases.

Key words: Oxidative stress, *Grewia villosa*, Root, Phytochemical, Free radicals.

1. Introduction

Now a days, Oxidative stress (OS) is very common among the world population (Monaghan *et al.*, 2009). OS is mainly due to the imbalance of the free radical and antioxidants in the body. Free radicals are unstable molecules with oxygen containing free electrons, which are ready to react with other molecules to stable. In this process they form long chain reactions because of their easy reaction with other molecules (Valko *et al.*, 2007). These reactions causing the damage to fatty tissues, proteins and DNA in the body (Richter *et al.*, 1988; Yu, 1994). The free radicals number in the body will increase because of over metabolism rate and low number of antioxidant to stabilize them. The presence of more number of free radicals will enhance the infections causes by the pathogens. By the over time this condition will damage the organs of the body in chronic diseases patients like diabetes, neurodegenerative diseases, heart diseases etc., (Halliwell and Gutteridge, 2007). As mentioned above, OS is common in people because of today's life style like fast foods, cigarette smoking, radiation, pollution, pesticides etc., So, there is a need to provide prevent worlds' population from OS by providing antioxidants either by naturally or in the form of modern drugs. In recent studies of different researchers, many medicinal plants have been using as antioxidant supplements around the world (Yashin *et al.*, 2017; Rajananda Swamy and Ganga Rao, 2018). In this regards, the present study was aimed to evaluate the antioxidant activity of *Grewia villosa*.

Grewia villosa is an medium sized tree belongs to the family Tiliaceae grows around Indian subcontinent. *G. villosa* has been using as fodder and antibiotic on different body ailments like skin diseases (Badami *et al.*, 2003). The stem bark paste employing to treat dysentery and wood in power form as an antidote to opium poisoning (Wali *et al.*, 2012). There was very few reports on scientific reports on *G. villosa* root parts, we collected the root parts of *G. villosa* and studied their phytochecmical analysis and antioxidant activity on different free radicals.

2. Materials and methods

2.1 Chemicals

The solvents used in the current research work were analytical grade. 1, 1-Diphenyl-

2picylhydrazyl (DPPH), Nitroblue tetrazolium, Riboflovin were purchased from Sigma chemicals, USA.

2.2 Collection of plant material and Preparation of extract

The plant material *Grewia villosa* was collected near Araku valley region, Visakhapatnam, Andhra Pradesh, India. The root part was separated from collected material and washed under running tap water and dried under room temperature. Then, plant material was made into coarsely powder and used for successive extraction with ethyl acetate and hydroalcoholic (70% Ethanol). The extracted solvents were dried under pressure in rota vapor.

2.3 Qualitative phytochemical analysis

The extracts of *G. villosa* were analyzed for identification of different phytochemicals in them using standard phytochemical qualitative tests (Veda Priya *et al*., 2018).

2.4 Antioxidant activity

Antioxidant activity of selected plant was evaluated using different free radicals. The extracts were dissolved in Dimethyl sulphoxide (DMSO) at different concentration and the experiments were repeated for three times, results were showed as mean ± SEM, % inhibition and IC_{50} values were calculated (Mallikarjuna Rao *et al.,* 2012).

2.4.1 Superoxide free radical scavenging activity

Superoxide free radical scavenging activity was carried out as per method described by the MeCord and Fridovich (1969). The method is mainly by measuring the absorbance at 560nm of antioxidants i.e., generation of superoxide free radical by riboflavin in the presence of nitroblur tetrazolium and their reduction by testing antioxidant or standard drug ascorbic acid.

2.4.2 Hydroxyl free radical scavenging activity

Hydroxyl free radical scavenging activity was measured using method said by Elizabeth and Rao, 1990. The method simply by measuring the extracts' absorbance at 532nm on generated hydroxyl free radical bys Fe+2/EDTA/H2O2 system (Fenton reaction).

2.4.3 DPPH free radical scavenging activity

The DPPH free radical scavenging activity was measured as method said by Braca *et al.,* 2003 and Anitha Murali *et al.,* 2011. The method based on color absorbance of DPPH solution in alcohol (purple) and after the addition of testing antioxidants (yellow). The change in color from purple to yellow indicated the presence of antioxidant activity to testing compounds.

2.4.4 Percentage inhibition and 50% inhibition concentration calculation

The percentage inhibition was calculated using below formula

Inhibitory ratio= (Absorbance of control-Absorbance of testing compound or ascorbic acid)/Absorbance of control × 100

The XY graph was plotted against tested extracts concentrations and their percentage inhibition was used to find 50% inhibition concentration.

3. Results and Discussion

3.1 Phytochemical analysis

The preliminary phytochemical analysis of *G. villosa* ethyl acetate and hydro-alcoholic extracts showed the variation in the presence and absence of tested phytochemical tests. The ethyl acetate extract showed the presence of steroids, terpenoids, alkaloids, phenols, glycosides, tannins, carbohydrates and absence of oils, amino acids, saponins, quinones, flavanoids. The hydro-alcoholic extract showed the presence of steroids, triterpenoids, phenols, alkaloids, glycosides, carbohydrates, flavanoids, tannins, saponins and absence for amino acids, quinones (Table 1).

Table 1. Phytochemical analysis of *Grewia villosa* extracts.

Phytochemical constituents	***Grewia villosa* extracts**	
	Ethyl acetate	**Hydro-alcoholic**
Phytosterols	+	+
Terpenoids	+	+
Glycosides	+	+
Saponins	-	+
Flavonoids	-	+
Tannins	+	+
Carbohydrates	+	+
Alkaloids	+	+
Amino acids	-	-
Oils	-	+
Phenols	+	+
Quinones	-	-

3.2 Antioxidant activity

The extracts of *G. villosa* exhibited concentration dependent reduction on tested free radicals i.e. hydroxyl, superoxide and DPPH and their protection was comparable with standard drug ascorbic acid.

The ethyl acetate and hydro-alcoholic extracts showed percentage inhibition on hydroxyl free radical vary 5.33% at 25 µg to 55.2% at 400 µg and 7.87% at 25 µg to 64.9% at 400 µg respectively. The ascorbic acid showed percentage inhibition on hydroxyl radical was 15.67 % at 5 µg and 84.23 % at 80 µg respectively (Fig 1).

The ethyl acetate and hydro-alcoholic extracts showed percentage inhibition on superoxide free radical vary 4.33% at 25 µg to 50.4% at 400 µg and 7.07% at 25 µg to 58.43% at 400 µg respectively. The ascorbic acid showed percentage inhibition on hydroxyl radical was 8.93 % at 5 µg and 74.6% at 80 µg respectively (Fig 2).

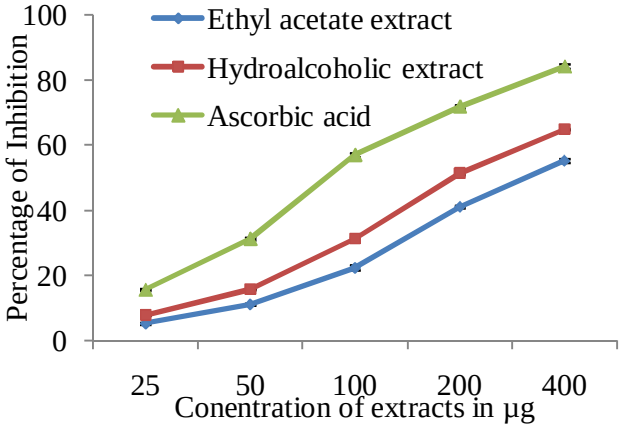

Fig 1. Percentage inhibition of ethyl acetate and hydro-alcoholic extracts of *Grewia villosa* on hydroxyl free radical.

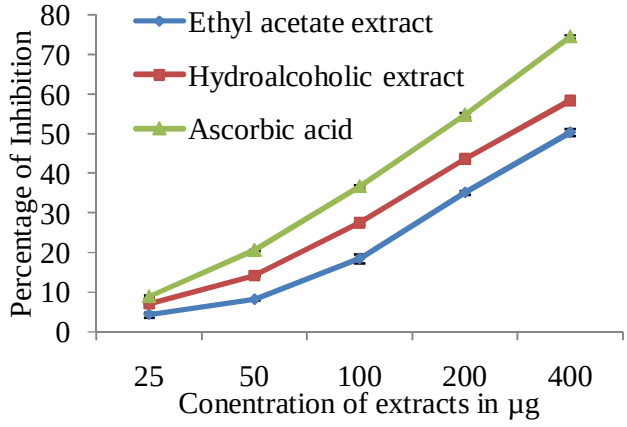

Fig 2. Percentage inhibition of ethyl acetate and hydro-alcoholic extracts of *Grewia villosa* on superoxide free radical.

The ethyl acetate and hydro-alcoholic extracts showed percentage inhibition on DPPH free radical vary 8.33% at 25 µg to 63.6% at 400 µg and 10.6% at 25 µg to 77.97% at 400 µg respectively. The ascorbic acid showed percentage inhibition on hydroxyl radical was 12.57 % at 5 µg and 86.5 % at 80 µg respectively (Fig 3).

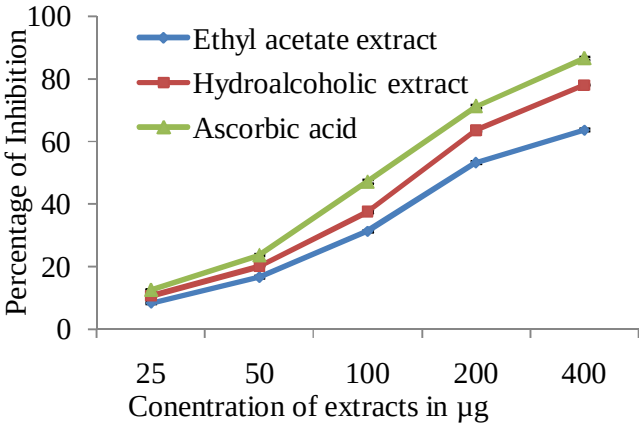

Fig 3. Percentage inhibition of ethyl acetate and hydro-alcoholic extracts of *Grewia villosa* on DPPH free radical.

The mean IC50 values for the ethyl acetate and hydro-alcoholic extracts on hydroxyl, and DPPH free radicals were 325 µg and 193 µg, 185 µg and 162 µg. The IC50 value for ethyl acetate extract on superoxide free radical was unable to found on tested range concentration of extract, however IC50 value for hydro-alcoholic extract was 304 µg. The IC50 value for ascorbic acid was 78 µg, 155 µg and 108 µg (Table 2).

Table 2. IC 50 values of *Grewia villosa* extracts on different free radicals.

Name of the plant/ compound	**Name of the extract**	**IC 50 value in µg on different free radicals**		
		DPPH	**Hydroxyl**	**Superoxide**
Grewia villosa	Ethyl acetate	185	325	N/a
	Hydro-alcoholic	162	193	304
Ascorbic acid	-	155	78	108

N/a: Not applicable

Different free radicals are generate during different metabolisms of the body. The usual levels of free radicals are not effecting the body but if their amount increase the body's antioxidant will reduce their number and normalize the body function (Yu, 1994). If the free radicals quantity raise in number, because of their natural reactive nature to stabilize,

they react with the cellular components causes metabolisms' imbalance and may finally leads to lipids peroxidation, DNA damage, neurodegenerative disorders and OS also leads to more aging compared to actual age (Storey, 1996). Therefore, there is a need to identify more antioxidants with fewer side effects and within economical. So, the present work was carried out to identify antioxidant nature of *G. villosa*. The root extracts of *G. villosa* showed the presence of different bioactive molecules in them like steroids, terpenoids, alkaloids, flavanoids etc., and showed concentration dependent percentage inhibition on different free radicals, this indicates the presence of antioxidant activity. The hydro-alcoholic extract showed the more activity compared to ethyl acetate extract and it showed more activity on DPPH and hydroxyl free radicals. The use of herbal medicine has been increasing since last decade because of their low side effects, easily availability and economical around the world. The present study confirms the antioxidant activity of *G. villosa* root part and it can be use as fit for human consumption for gaining the antioxidants.

Conclusion

Oxidative stress is the one of the main condition in the body to boost diseases because of their chain reactions with cellular components. The antioxidants will stabilize oxidant by reacting with them instead of cellular components. The current research confirms antioxidant activity of *Grewia villosa* root extracts and further research is worth full to identify responsible bioactive compounds from these extracts on reduction of oxidative stress and other harmful diseases.

Acknowledgements

The authors are thankful to Department of Botany, College of Science and Technology, Andhra University for providing the necessary facilities for current research work.

Conflict of interest

None to declare.

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