Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2012, 4 (2):525-529
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

An antioxidant and Anti bacterial activity of *Dregea volubilis* leaves extract

Purushoth Prabhu.T[1*], Maheswaran.V. S[2], Selvakumari. S[1], Suriyapadminimoka[2], Ragadeepthi. S[2] and Guduvalli Dileep[2]

[1]*Dept of Pharmacognosy, C. L. Baid Metha College of Pharmacy, Chennai*
[2]*Dept of Pharmaceutical chemistry, C.L.Baid Metha College of Pharmacy, Chennai*

ABSTRACT

The ethanolic extract of Dregea volubilis, benth was screened for anti oxidant and anti bacterial activity. Safer anti oxidants suitable for long term use are needed to prevent or stop the progression of free radical mediated disorders such as arthritis, hemorrhagic shock, diabetes, hepatic injury, aging neuro degenerative diseases and carcinogenesis. Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that anti oxidants reduce the risk for chronic diseases including cancer and heart diseases. The main characteristic of an anti oxidant is its ability to trap free radicals. Anti oxidant activity of ethanolic extract of Dregea volubilis were assessed using DPPH, hydroxyl, hydrogen peroxide, determination of reducing power, superoxide radical, free radical scavenging effects at various concentrations by reducing power assay. Thus augmenting the wide use of plant in the indigenous system of medicine for free radical mediated disease. The invitro anti bacterial activity shows the potent inhibitory action against Staphylococcus aureus, Pseudomonas aeuruginosa, which causes carbuncles and dermatitis , with the pace of advancement in science and technology, there has been spectacular public health success with regard to control of various microbial infections. Therapeutic measures in the form of specific treatments through anti microbial drugs viz., antibiotics, anti viral, and anti fungal agents are effective in controlling the diseases. From the above studies the plant exhibits high potent anti oxidant, anti bacterial activity and the drug can be recommended for minor infectious diseases.

Key words: *Dregea volubilis*, Anti oxidant, Anti bacterial, DPPH.

INTRODUCTION

Current research is now directed towards naturally occurring anti oxidants of plant origin [1]. An anti oxidant is a molecule capable for inhibiting the oxidation of other molecules [2]. Oxidation reactions can produce free radicals; these radicals can start chain reactions[3]. Anti oxidants can interfere with the oxidation process by reacting with free radicals, chelating metals, and also by acting as oxygen scavengers[4]. Anti oxidants have been reported to prevent oxidative damage by free radical and reactive oxygen species (ROS)[5] and may prevent the occurrence of disease including brain disorders, cancer, atherosclerosis, inflammatory disease and variety of other disorders[6,7]. The oxidative stress may constitute the key and common event in the pathogenic conditions. Free radicals are continuously produced in the body as a result of normal metabolic processes and interaction with environmental stimuli[8]. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent anti oxidant activities, no side effects and economic viability[9,10]. The genus Dregea volubilis (family: Asclepidaceae) occur in the warmer regions. The plant leaves were used in eye

infections and for health improvement. Leaves are mostly employed as an application to boils and abscesses, roots and tender stocks are considered as emetic and expectorant.

Microbial pathogens are responsible for more than 14 million human deaths per annum[11]. In last quarter of 20th century, a large number of microbial pathogens have merged and re-emerged in different parts of globe and majorities (75%) of these pathogens are known to be zoonotic[12]. The prophylactic measures for most of the important diseases are far from expectation due to non-availability of effective vaccines. Therefore therapeutic measures in the form of specific treatments through anti microbial drugs such as antibiotics, anti viral and anti fungal agents are effective in controlling the diseases with promising perspectives. Through the plant has great potential for anti oxidant and anti bacterial activity, nobody has not been yet recommended these activities on the leaves of this plant[13]. In continuation of our earlier reports to provide information on anti oxidant for isolation and characterization, of active principles responsible for exerting the tested pharmacological uses.

MATERIALS AND METHODS

Chemicals used in this study were 1,1-diphenyl-2-picryl Hydrazyl (DPPH) , ethanol, hydrogen peroxide, ferric chloride, EDTA, ascorbic acid, P-NDA in phosphate buffer, hexane, chloroform. All reagents used for the study were analytical grade. Leaves are opposite and discontinuous, simple, laminate broadly ovate or sub orbicular, ,margins entire, flowering period will be may June, and fruiting period June-September. In Haryana the leaves were mostly used as anti inflammatory, anti microbial, anti pyretic. The leaves are collected, shade, dried, coarse powder and blender. The coarse powder was extracted using soxhlet extractor for 18hrs. Fresh leaves of Dregea volubilis are collected from Chennai, Tamil nadu and further identity was confirmed by tallying with herbarium specimens at the plant anatomy research center, by Prof. Jay Raman, Tambaram, Chennai, Tamilnadu.

Preparation of Extract

The extract was prepared by hot percolation method. The leaves were shade dried, coarse powder in a soxhlet extractor with hexane and chloroform using soxhlet extract for 18hrs. The solvent was removed by distillation using water bath and remaining under reduced pressure and dried in vaccum desiccators. The different extracts of Dregea volubilis were screened for antioxidant and antibacterial activity.

Antioxidant activity

DPPH free radical scavenging activity

The hydrogen donating ability of alcoholic extract was examined in the presence of DPPH ethanol solution was added to 2.5ml of sample solution of different concentration and allowed to react at room temperature. After 30 min the absorbance values were measured at 517nm ethanol (1ml) plus plant extract solution was used as a blank. DPPH solution (1 ml, 0.3 nm)plus ethanol (2.5ml) served as negative control. The positive control was those using the standard (ascorbic acid) solutions[14].

$$\% \text{ Anti- radical activity} = \frac{\text{Control Abs-Sample Abs}}{\text{Control Abs}} * 100$$

Scavenging of 2,2'-azino-bis (3-ethyl benzo thiazole-6-sulphuric acid) di ammonium salt (ABTS)radical cation

To 0.2ml of various concentrations of the extract or solution, 1ml distilled DMSO and 0.16ml of ABTS solution (2mM) were added and incubated for 20 mins. Absorbance of the solutions were measured spectrophotometrically at 734nm.

Free radical scavenging using hydrogen peroxide

A solution of hydrogen peroxide (20mM) was prepared in phosphate buffer saline (PBS) (Ph 7.4) . various concentrations of the extract or standard in methanol (1ml) were added to 2 ml of hydrogen peroxide solution in PBS. After ten minutes the absorbance was measured at 230nm[15].

Scavenging of Hydroxy Radical in the Para-Nitraso Di methyl aniline (P-NDA)

To the solution containing ferric chloride (0.1mM, 0.5ml) ascorbic acid (0.1ml of 0.5ml) H_2O_2(2mM of 0.5ml)P-NDA(0.01mM of 0.5ml) in phosphate buffer(P^H7.44,20mM) were added. Various concentrations of the extract or

standard in distilled DMSO or dissolving solvent or alcohol (0.5ml) to produce a final volume of 3ml.Absobance was measured at 440nm.

Anti bacterial activity
Preparation of media
Nutrient agar media was used for cultivation of bacteria and particularly pathogenic bacteria which is associated with disease.

The various bacterial organisms used in the present study include:
Gram positive bacteria- *Staphylococcus aureus*
Gram negative bacteria- *Escherichia coli, Pseudomonas aeruginosa*

Preparation of agar plates with extracts
The extracts were introduced aseptically into sterilized petridishes to get final concentration ranging from 33.33µg, 66.66µg, 100perµl (0.5ml, 1ml and 1.5ml) and the volume was made up to 15ml by adding nutrient agar media and the petridishes were swirled until the agar begins to set.

Test Procedure
The plates with different extracts of various dilution were inoculated with a loopful of the cultures at the tabulated spots. These plates were incubated at 34^0c for 24hrs. The results were read by the presence or absence of growth of the organisms.

Zone of inhibition
The discs of 6mm diameter were prepared from whatmann filter paper no.1 and were sterilized in a hot air oven at 16^0c for 1hr. The discs were then impregnated with the extracts and the solvent Dimethyl formamide (DMF). Ciprofloxacin discs were used as standard. Each disc of ciprofloxacin contained 5µg. The pathogenic strains were then seeded as the nutrient agar media in a petridish by streaking the plate with the help of sterile swab. Care must be taken, seeded plates are allowed to dry and then the ciprofloxacin, extract and DMF discs were placed on the seeded medium plates and maintained at 4^0c for 30min to allow perfuram of drugs being tested. Plates are incubated at 37^0c for 24hrs. The results were observed by the presence or absence of zone of inhibition. The zone of inhibition was measured.

Statistical analysis
The experimental data were expressed as mean ± SEM and as percentage. The significance of difference among the various treated groups were analysed by one way ANOVA-Dunnetts multiple comparison test using Graphat Instant software.

RESULTS AND DISSCUSION

Antioxidant activity
Anti oxidants exert their mode of action by suppressing the formation of reactive oxygen species either by inhibition of enzymes or by chelating trace elements which are involved in free radical generation, scavenging reactive species and up regulating or protecting anti oxidant defences.

DPPH free radical scavenging activity
DPPH radical was used as a substrate to evaluate free radical scavenging activities of Dregea volubilis extract. It involves reaction of specific anti oxidant, with a stable free radical which produces a deep violet colour containing 1,1-diphenyl-1,2-picryl hydrazyl (DPPH). As a result, there is reduction of DPPH concentration by anti oxidant, which decreases the optical absorbance of DPPH, there is detected by spectrophotometer at 517nm. The reduction in the number of molecules can be correlated with the number of available hydroxyl radicals. The good activity of the extract may be probably due to the presence of substances with on available hydroxyl group.

Scavenging of 2,2[1]-azino-bis (3-ethyl benzo thiazoline-6-sulphuric acid) diammonium salt(ABTS) radical cation

In the ABTS method, the hexane and chloroform extract of Dregea volubilis showed potent anti oxidant activity with IC $_{50}$ values ranging from 13.26 to 24.36µg/ml. However the standard rutin and ascorbic acid exhibited better results with lower IC_{50} values.

Free radical scavenging using Hydrogen peroxide

In H_2O_2 method the chloroform extract showed potent activity greater than or comparable to the standard rutin.

Scavenging of Hydroxyl radical in the Para-Nitraso-Dimehyl aniline (P-NDA)

In the P-NDA method the extracts showed potent or comparable anti oxidant activity to those of the standards used. Based on the various in vitro assays, the Dregea volubilis leaf extract possess strong anti oxidant activity, evidenced by the free radical scavenging activity. The free radical scavenging activity of the extracts were evaluated based on the ability to scavenge the synthetic DPPH. This assay provided useful information on the reactivity of the compounds with stable free radicals, because of the odd number of electrons. The decrease in absorbance at 560nm with anti oxidants indicates the consumption of reaction mixture.

Table1 Anti-oxidant activity of the hexane and chloroform extracts by different models

50% inhibitory concentration-IC_{50}(mg/ml)				
Drug	DPPH	ABTS	H_2O_2	P-NDA
Hexane extract	90.22±1.32	24.36±0.29	139.06±1.37	60.85±1.46
Chloroform extract	12.41±0.76	13.86±1.33	40.07±0.65	25.71±2.31
Ascorbic acid	10.52±1.45	18.48±0.56	12.3±0.73	1.78±0.86
Rutin	5.42±2.06	0.52±0.05	45.28±0.75	20.45±9.33

Mean±SEM

Anti bacterial activity

The experiment to find the zone of inhibition was carried out with the hexane and chloroform extracts of Dregea volubilis, after calculating the minimum inhibitory concentration against the pathogenic bacteria. The chloroform extract shows potent inhibitory activity at the low dose of 66.66µg/ml compared to hexane extract. The plant Dregea volubilis was used in siddha system of medicine for the treatment of eye disorders. The invitro anti bacterial activity shows the potent inhibitory action against Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae which causes carbuncles and dermatitis. This confirms that the plant exhibits inhibitory action against eye infection caused by pathogenic bacteria.

Table-2 Minimum Inhibitory Concentration for Anti bacterial activity

S.No.	Extracts	Conc.µg/ml	S.a	E.c	K.p	P.a
1	Hexane	33.33	+	+	+	+
		66.66	+	-	+	+
		100	-	-	-	-
2	Chloroform	33.33	+	+	+	+
		66.66	+	+	+	+
		100	-	-	-	-

+showed the presence of growth
-Absence of growth of organism
S.a→ Staphylococcus aureus, E.c → Escherichia coli, K.p→ Klebsiella pneumoniae
P.a→ Pseudomonas aeruginosa

Table-3 Zone of Inhibition(in mm) of various extracts

Extract	***E.Coli***	***Klebsiella***	***Pseudomonas***	***S.aeruginosa***
Hexane	13	10	7	7
Chloroform	11	9	8	11
Ciprofloxacin	35	32	30	30

CONCLUSION

The Chloroform extract of *Dregea volubilis* possesses good anti oxidant, anti bacterial activity. So, the further investigation the study can be continued for isolation and characterization of active principles responsible for exerting the tested pharmacological uses is needed.

REFERENCES

[1] Nagendran CG(**2005**) *Reasonce* 10(2):65-73.
[2] Sies, Helmut (**1997**). *Experimental physiology* **82** (2): 291–5.PMID 9129943.
[3] Werner Dabelstein, Arno Reglitzky, Andrea Schütze and Klaus Reders "Automotive Fuels" in Ullmann's Encyclopedia of Industrial Chemistry **2007**, Wiley-VCH, Weinheim.doi:10.1002/14356007.a16_719.pub2.
[4] Trenam CW; Blake DR; Morris CJ.*J Invest Dermatol*,**1992**,99,675-82.
[5] Nagendran CG(**2005**), *Resonance* 10(2):65-73.
[6] Halliwell B, Gutteridge JMC(**1999**) Free readicalin biology andmedicine.3rd ed.London:Oxford.p.36-40,311-12.
[7] Daniel, R.S., Mathew, B.C., Devi, KS., **1998**. *Indian J Exp Bio* 36;902-906.
[8] Baillie, J.K.; Thompson, A.A.R.; Irving, J.B.; Bates, M.G.D.; Sutherland, A.I.; MacNee, W.; Maxwell, S.R.J.; Webb, D.J. (**2009**). *QJM***102** (5): 341–8. doi:10.1093/qjmed/hcp026. PMID 19273551.
[9] Agarwal A, Prabhakaran SA(**2005**) *Indian J Exp Biol* 43:963-974.
[10] Badami, S., Om Prakash., Dongre, S.H. and Suresh.B (**2005**) *Indian J. Pharmacol.*, 37B, 251-252.
[11] Pankaj Chaudhary, Sandeep Kumar Shukla and Rakesh Kumar Sharma, **2006**. A Fractionated Extract of *Podophyllum hexandrum* Protects Cellular DNA from Radiation-induced Damage by Reducing the Initial Damage and Enhancing Its Repair *in vivo*. Evidence based Complementary and Alternative Medicine(e CAM).
[12] Soares JR. Dinis TCP, CunhaA, Almeida LM. *Free Rad Res*, **1997**, 26:469-78.
[13] Badami, S., Om Prakash., Dongre, S.H. and Suresh.B (**2005**) *Indian J. Pharmacol.*, 37B, 251-252.
[14] Kerri, E. and Rao, M.N.A. (**1990**) Oxygen radical scavenging activity of curcumin. *Ind J. Pharm*., 58, 237-240.
[15] Ecobichon DJ. The Basis of Toxicology Testing. CRC Press, New York, **1977**, 43-86.