International Journal of Current Trends in Pharmaceutical Research

Journal Home Page: www.pharmaresearchlibrary.com/ijctpr

Research Article Open Access

Evaluation of *In-vitro* Antioxidant Activity of Ethanolic Extract of *Terminalia Arjuna* Leaves

B. Aruna[1]*, R. Venu Madhuri[1], Dr. D. Madhuri[1], Dr. A.V. Badrinath[1], Sk. Karimulla[2]

[1]*Dr. K.V. Subbareddy Institute of Pharmacy, Dupadu, Kurnool, Andhra Pradesh, India-518218.*
[2]*Department of Pharmacology, Narayana Pharmacy College, Chinthareddy Palem, Nellore, Andhra Pradesh-524003*

A B S T R A C T

Arjuna (Terminalia Arjuna) is a widespread medicinal plant used in the Ayurvedic system of medicine to cure for various ailments and is one of the active ingredients in numerous polyherbal hepatoprotective formulations used in India. Leaves possess glycosides, large quantities of flavonoids, tannins and minerals. Flavonoids have been found to possess antioxidant, anti-inflammatory and lipid lowering effects. This work was carried out to investigate the antioxidant activity and free radical scavenging capacity of leaf extract of terminalia arjuna prepared in ethanol. The extraction yield of extract was found to be 17.14gm on dry weight basis. From the DPPH radical scavenging activity, phosphomolybdenum method and hydrogen peroxide scavenging method, it can be concluded from the results that arjuna extract was good source of natural antioxidants.

Keywords: antioxidant activity, terminalia arjuna, phosphomolybdenum, hydrogen peroxide

A R T I C L E I N F O

CONTENTS

1. Introduction 86
2. Materials and Methods 87
3. Results and discussion 88
4. Conclusion 88
5. References 89

Article History: Received 10 January 2017, **Accepted** 28 February 2017, **Available Online** 15 March 2017

***Corresponding Author**
B. Aruna
Dr. K.V. Subbareddy Institute of Pharmacy, Dupadu, Kurnool, Andhra Pradesh, India-518218.
Manuscript ID: IJCTPR3347

PAPER-QR CODE

Citation: B. Aruna, *et al.* Evaluation of *In-vitro* Antioxidant Activity of Ethanolic Extract of *Terminalia Arjuna* Leaves. *Int. J. Curnt. Tren. Pharm, Res.*, 2017, 5(2): 86-89.

1. Introduction

The reactive oxygen species (ROS) including superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide are often generated as byproducts of biological reaction ROS play an important role in cell metabolism including energy production, phagocytosis and intracellular signaling however, these ROS produced by sunlight, chemical reactions and metabolic processes have a wide variety of pathological effect such as DNA damage,

carcinogenesis and various degenerative disorders such as cardiovascular diseases ageing and neuro-degenerative diseases. The antioxidants are molecules capable of decreasing or preventing the oxidation of substrate molecules. Terminalia arjuna (telugu name; thellamaddi, English name: Arjuna myrobalam) from combretaceae family is a large tree which is found throughout the south asian region. This tree is usually an evergreen tree with new leaves appearing in the hot season (February to April) before leaf fall. Termanalia Arjuna is a good hypocholosteremic, hypolipidemic, anticoagulant, antihypertensive, antithrombotic, antiviral, antifungal and anti bacterial agent. Many useful phytoconstituents have been isolated from termanalia Arjuna which includes, triterpenoids for cardiovascular properties, tannins and flavanoids for its anticancer properties, and so on. The bark leaves and fruits of termanalia Arjuna have been used in indigenous system of medicine for different elements. Terminalia Arjuna bark contains a very high level of flavonoids compared to other commonly used plant item. Medicinal plants containing active chemical constituents with high antioxidant property play an important role in the prevention of various degenerative diseases. phenolics are commonly found in medicinal plants and have been reported to have multiple biological effects, including anti oxidant activity.

T.arjuna contains phenols, flavonoids, tannin, saponin, alkaloids, glycosides, phytosterols and carbohydrate. Most of the antioxidant compounds in a typical balanced diet are derived from plant sources with a wide variety of biological and chemical properties. Synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used as food additives, but recent reports have expressed safety concerns allowing natural antioxidant to be the focus of intense interest (sun and HO,2005;wilson,1999). Other contributors to the antioxidant activity include alkaloids, proteins, minerals and other vitamins such as the carotenoids and vitamin B6, B12 and K (smolin and Grosvenor, 2007). Antioxidant compounds like phenolic acids, polyphenols and flavonoids scaveng free radicals such as peroxide, hydroperoxide of lipid hydroxide and thus inhibit the oxidative mechanisms that lead to degenerative diseases (subramanion et al., 2011). There are number of clinical studies confirming the powerful anti cancerous and anti cardiovascular properties of poly phenols [parkas et al, .2007; bajpai et al, .2005; sidduraju and Becker, 2003].

2. Materials and Methods

Plant material collection:

Termanalia arjuna leaves were collected from vijaya park in kurnool. Leaves were dried under the shade for 3-4 days and then powdered.

Soxhlet extraction:

Soxhlet extraction is only required when the desired compound has a limited solubility in a solvent, and the impurities are insoluble in that solvent. Termanalia arjuna leaves were extracted in soxhlet apparatus using ethanol as solvent.

Phytochemical analysis:

Phytochemicals analysis for major phytocontituients of the plant extract was undertaken using standard methods. The plant was screened for the presence of biologically active compounds like sugars, amino acids, proteins, phenols, terpenoids etc.

***In-Vitro* Antioxidant Activity:**

DPPH [2,2-diphenyl 1-picrylhydrazyl free radical scavenging assay]: The free radical scavenging capacity of the extracts was determined using DPPH. Freshly prepared DPPH solution was taken in test tubes and extracts were added followed by serial dilutions to every test tube so that the final volume was 5 ml and after 30 min, the absorbance was read at nm using a spectrophotometer. Ascorbic acid and BHT were used as standard. Control sample was prepared containing the same volume without any extract and standard, and the absorbance read at 580nm using a spectrophotometer. Ethanol was served as blank. % inhibition of DPPH free radical was measured using the following equation.

% Inhibition = $(1- A_{1/}A_0)\times 100$

Where, A =absorbance of extract or standard and

$A_{0 =}$ Absorbance of the control.

Hydrogen peroxide scavenging assay:

Scavenging activity of extract and its sub-fractions were evaluated by hydrogen peroxide (jayaprakasha et al. 2004). 1ml of various concentrations of the extract, sub-fraction standards in ethanol was added to 2ml of hydrogen peroxide solution in phosphate buffered saline (PBS, P^H 7.4). Then finally the absorbance was measured at 230 nm after 10 min. Ascorbic acid and BHT were used as standard. Control sample was prepared containing the same value without any extract and standard and the absorbance read at 230 nm using a spectrophotometer. The percentage inhibition was calculated according to the following equation:

% Inhibition= $(1-A_{1/}A_0) \times 100$

Where, A_0= absorbance of the control and

A_1= absorbance in the presence of the sample and extract and standard.

Phosphomolybdenum method:

Total antioxidant capacity assay is a spectroscopic method for the quantitative determination of antioxidant capacity, through the formation of Phosphomolybdenum complex. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate Mo (V) complex at acidic P^H. Total antioxidant capacity can be calculated by the method described by prieto et al.(1999). 0.1 ml of sample (100µg) solution is combined with 1ml of reagent (0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tube is capped and incubated in a boiling water bath at 95^0c for 90 min. After cooling the sample to room temperature, the absorbance of the aqueous solution is measured at 695 nm against blank in UV-Spectrophotometer. A typical blank solution contained 1ml of reagent solution and the appropriate volume at the same solvent used for the sample and it is incubated under same conditions as rest of sample. For the samples of unknown composition, antioxidant

capacity can be expressed as equivalents of alpha-tocopherol.

3. Results and Discussions

Preliminary phytochemical screening: Qualitative phytochemical investigation of crude plant extract of Terminalia *arjuna* (greenish black colour) revealed the presence of alkaloids, carbohydrates, starch, protein, amino-acid, steroids, flavanoids, and absence of starch**.**

Table 1

S.No	Phytoconstituent	Test	Results
1	carbohydrate	Molish test	+VE
2	starch	Iodine	-VE
3	protein	Millons test	+VE
4	Amino-acid	Cysteine test	+VE
5	steroid	Salkowski test	+VE
6	flavanoids	Ferric chloride	+VE
7	Alkaloids	Mayers test	+VE
8	Tannins and phenolic compounds	5% $FeCl_3$ test	+VE
9	Oxalic acid	Calcium chloride	+VE
10	Inorganic acid	Sulphate test	+VE

In-Vitro Anti Oxidant Study

DPPH free radical scavenging assay

Free radical scavenging activity was evaluated using stable DPPH free radical (2-2 diphenyl 1-picrylhydrazyl).activity of extract increase in concentration of phenolic compounds. Lower the IC_{50} valus of extract, more effective it will be for inhibition of DPPH free radicals and vice versa. Ethanolic T. arjuna leave extract showed a varied potential of antioxidant capacities in term of IC_{50} (µg/mL) ranging from 2.71-7.68 µg/mL .the lowest percentage inhibition of 80 % ethanolic leaf extract.

Table 2

Extract	% Inhibition (mg/g, AAE)
T.arjuna	0.675
α – tocopherol	0.7901

Scavenging of hydrogen pereoxide

Hydrogen peroxide, although not a radical species play a role to contribute oxidative stress. Naturally –occurring iron complexes inside the cell believed to react with H_2O_2 In vivo to generate highly reactive hydroxyl radicals and this may be the origin of its toxic effects. Scavenging of hydrogen peroxide of extract of ethanol in highest position in depleting H_2O_2.

Scavenging of hydrogen pereoxide

Hydrogen peroxide, although not a radical species play a role to contribute oxidative stress. naturally –occurring iron complexes inside the cell believed to react with H_2O_2 In vivo to generate highly reactive hydroxyl radicals and this may be the origin of its toxic effects. Scavenging of hydrogen peroxide of extract of ethanol in highest position in depleting H_2O_2.

Table 3

Extract	% Scavenging activity (mg/g, AAE)
T.arjuna	0.53
α –tocopherol	0.79

Determination of total antioxidant capacity:

Total antioxidant capacity of the different extracts of T.arjuna was evaluated by the phosphomolybdenum method and was expressed as ascorbic acid equivalents (AAE) per gram of plant extract. Total antioxidant capacity of the test samples was calculated using the standard curve of ascorbic acid equivalents (AAE) per gram of plant extract. Total antioxidant capacity of the test samples was calculated using the standard curve of ascorbic acid ($y=0.002\times +0.001$:$R^2=0.997$) ethanolic.

Table 4

Extract	Total antioxidant capacity (mg/g, AAE)
T. arjuna	0.3776
α –tocopherol	0.7528

Discussion

In this study the anti-oxidant activity of Terminalia arjuna leaf extract was compare to α-Tocopherol. The anti oxidant activity was evaluated in a series of the following invitro tests, i.e.,DPPH scavenging activity, Hydrogen peroxide scavenging activity and Total anti oxidant capacity (Phosphomolybdenum method). The result of DPPH scavenging ability,in this study indicates that the plant was potentially active. This suggests that the plant extract contain compounds that are capable of donating Hydrogen to a free radical inorder to remove odd electron which is responsible for the radicals activity followed by ethanolic extract. Hydrogen peroxide is a week oxidizing agent and it is not very reactive, can cross biological membranes. Because of the possible involvement of H2O2 in the generation of hydroxyl radicals, this property places hydrogen peroxide in a prominent role to intiate cytotoxicity than its chemical reactivity. Thus, removing H2O2 is very important for the protection of living systems. T. arjuna scavenged hydrogen peroxide which may be attributed the presence of phenolic groups that could donate electrons to hydrogen peroxide thereby neutralizing it into water.

4. Conclusion

On the basis of results obtained in the present study it was concluded that the ethanolic extract of Terminalia arjuna posses significant anti oxidant activity compare to α-Tocopherol.

5. References

[1] Inhibition of Kupffer cell functions as an explanation for the hepatoprotective properties of silibinin. Dehmlow, Carola; Erhard, Jochen; De Groot, Herbert. *Hepatology* (1996), 23(4), 749-54.

[2] Jia X.-Y., Zhang Q.-A., Zhang Z.-Q., Wang Y., Yuan J.-F., Wang H.-Y., Zhao D."Hepatoprotective effects of almond oil against ccl4_induced liver injury in rats" food chemistry 2011 125:2 (673-678)

[3] Wang J.-H., Shin J.-W., Kim H.-G., Hye-Jung P., Choi M.K., Son C.-G."Hepatoprotective and antioxidant effects of ethyl acetate soluble fraction of Amomum xanthoides Hepatology International 2010 4:1 (281-282)

[4] Lin S.C., Lin C.H., Lin C.C., Lin Y.H., Chen C.F., Chen I.C., Wang L.Y. Hepatoprotective effects of Arctium lappa Linne on liver injuries induced by chronic ethanol consumption and potential ccl4. Journal of biomedical science 2002 9:5 (401-409)

[5] Chien C.-F., Wu Y.-T., Tsai T.-H."Biological analysis of herbal medicines used for the treatment of liver diseases"biomedical chromatography_2011 25:1 (21-38)

[6] Tabassum N., Qazi M.A., Shah A., Shah M.Y. Curative potential of Cashing (Cichorium intybus Linn.) extract againstccl4 induced hepatocellular damage in rats pharmacologyonline 2010 2 (971-978)

[7] Sarkar K., Sil P.C. Attenuation of acetaminophen-induced hepatotoxicity in vivo and in vitro by a 43-kD protein isolated from the herb Cajanus indicus L Toxicology Mechanisms and Methods 2007 17:6 (305-315

[8] Manna P., Sinha M., Sal P.C. A 43 kD protein isolated from the herb Cajanus indicus L attenuates sodium fluoride-induced hepatic and renal disorders in vivo Journal of Biochemistry and Molecular Biology 2007 40:3 (382-395)

[9] Haleagrahara N., Chakravarthi S., Kumar P. A study of protective effect of centella asiatica in 1-methyl-4phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)-induced liver injury Haleagrahara N., Chakravarthi S., Kumar P. Hepatology International 2009 3:1 (196-197)

[10] Rao G.M.M., Vijayakumar M., Rao Ch.V., Rawat A.K.S., Mehrotra S. Hepatoprotective effect of Coccinia indica against CCl4 induced hepatotoxicity Natural Product Sciences 2003 9:1 (13-17)

[11] Steinkellner, H.; Rabot, S.; Freywald, C.; Nobis, E.; Scharf, G.; Chabicovsky, M.; Knasmüller, S.; Kassie, F. (2001). "Effects of Cruciferous Vegetables and their Constituents on Drug Metabolizing Enzymes Involved in the Bioactivation of DNA-Reactive Dietary Carcinogens". *Mutation Research* 480–481: 285–297.

[12] XuR.-M., Deng K.-M., Lu Y.,"Studies on strengthening and restoring effects and hepatoprotective effect of Eclipta" Journal of shanghai university (Medical Science) 2009 29:10 (1200-1204.