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Evaluation of *in-vitro* antioxidant activity of some medicinal *herbal plants* leaves extracts

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Abstract— *Free radicals are atoms or molecules or ions that have unpaired valence electron, usually unstable and highly reactive Over expression of free radicals exposes the tissues to conditions of oxidative stress. Oxidative stress is believed to be a primary factor in various chronic and degenerative diseases such ascancer, atherosclerosis, gastric ulcers, diabetes, Alzheimer's disease, Parkinson's disease, aging and other conditions.The Persea americana, Phoenix dactylifera, Syzygium cumini and Vitis vinifera leaves selected in this study are inedible, generally discarded as waste and underutilized resource in food industry had shown significant antioxidant activity.*

Keywords— *Persea americana, Phoenixdactylifera, Syzygium cumini, Vitis vinifera, Antioxidant*

I. INTRODUCTION

Oxidative stress (OS) is the root cause of several diseases. OS represents an imbalance between reactive oxygen species (ROS) production and the cellular antioxidant defense system. In stress condition, ROS levels increase and cause cell damage, necrosis and apoptosis via oxidation of lipids, proteins and DNA (Elahi M. M *et al.*, 2009). Oxidative stress can induce lipid peroxidation and form lipid peroxides, such as malodialdehyde and 4-hydroxy-2-nonenal (4-HNE) involved in cell membrane destabilization and causing hepatic cell necrosis. The role of ROS and other free radicals (FR) in disease pathology is well established and they are known to be involved in many acute and chronic disorders, such as aging, diabetes, atherosclerosis, cancer etc. (Harman D, 1998)

II. MATERIALS AND METHOD

Tris-HCl, potassium chloride, ammonium ferrous sulfate, sodium dodecyl and acetic acid were purchased from S.D Fine chemicals, Mumbai.Acetic acid and thiobarbituric acid were purchased from Lobachemie, USA.

Lipid peroxidation inhibition was determined by the method developed by Ohkawa (Ohkawa H *et al.*, 1979). Rat liver weighing 10 g was homogenized with a polytrion homogenizer in ice cold Tris-HCl buffer to produce 25% w/v homogenate.It was centrifuged at 4000 rpm for 10 min.,an aliquot of 0.1 ml supernatant was mixed with 0.1 ml of the leaves extract of different concentrations, followed by addition of 0.1 ml of potassium chloride (10 mM), 0.1 ml of ascorbic acid (0.06mM) and 0.1 ml of ammonium ferrous sulphate (0.16mM) and were incubated for one hour at 37^{o}c. The reaction mixture was treated with 0.2 ml of sodium dodecyl sulphate (8.1%), 1.5 ml ofthiobarbituric acid (0.8%) and 1.5 ml of 20% acetic acid (pH 3.5). The total volume was made upto 4 ml by adding distilled water and kept in an oil bath at 100^{o}c for 1 hour. Afterthe mixture was cooled, 1 ml of distilled water and 5 ml of 15:1 v/v butanol-pyridine mixture was added. Following vigorous shaking, the tubes were centrifuged at 4000 rpm for 10 min and the absorbance of the organic layer containing the thiobarbituric acid reactive substance (TBARS) was measured at 532 nm. A control was prepared using 0.1 ml of vehicle in the place of leaves extract/ascorbic acid. The percentage inhibition of lipid peroxidation by the leaves extract was determined by comparing the absorbance values

of the control with the test using following formula.

Calculation of percentage inhibition

Percentage inhibition = $(A_0 - A / \mathbf{A_0}) \times 100$

A_0 =Average absorbance of control

A = Average absorbance of leaves extract

Calculation of 50% inhibition concentration:

The econcentration of the leaves extract/ascorbic acid required to inhibit lipid peroxidation by 50% (IC_{50}) was obtained by linearregression analysis of dose response curve plotting betweenpercentage of inhibition and concentrations.

III. RESULTS AND DISCUSSION

Lipid peroxidation inhibition activity:

The leaves extracts of *Persea americana, Phoenix dactylifera, Syzygium cumini, Vitis vinifera* and ascorbic acid at different concentrations (12.5-400 µg/ml)

inhibited the lipid peroxidation in a dose dependent manner (Table No. 1). The amount of leaves extract required to produce the 50% inhibition of lipid peroxidation was found tobe (µg) 82.46, 91.12, 279.64, 140.77 and 60.56 respectively.

The lipid peroxidation inhibition activity of leaves extracts and ascorbic acid was in the following order**:**

Ascorbic acid > *P.americana* > *P.dactylifera* > *V.vinifera* > *S.cumini* (Table No. 1)

Table No.1: Lipid peroxidation inhibition by selected leaves extracts & ascorbic acid in in vitro studies

Leaves extracts & AA	**Quantity (µg)**					
	12.5	**25**	**50**	**100**	**200**	**400**
	(%Inhibition)	**(%Inhibition)**	**(%Inhibition)**	**(%Inhibition)**	**(%Inhibition)**	**(%Inhibition)**
P.amerecana	0.181 ± 0.01	0.191 ± 0.002	0.23 ± 0.01	0.263 ± 0.01	0.311 ± 0.008	**0.36 ± 0.009**
	16.02	22.43	47.43	68.58	99.35	**130.76**
P.dactylifera	0.173 ± 0.005	0.184 ± 0.003	0.242 ± 0.01	0.279 ± 0.01	0.281 ± 0.007	**0.336 ± 0.02**
	10.89	17.94	55.12	78.84	80.12	**115.38**
S.cumini	0.163 ± 0.001	0.178 ± 0.002	0.188 ± 0.001	0.199 ± 0.005	0.223 ± 0.01	**0.256 ± 0.02**
	4.48	14.1	20.51	27.56	42.94	**64.1**
V.vinifera	0.167 ± 0.002	0.179 ± 0.003	0.192 ± 0.002	0.232 ± 0.02	0.292 ± 0.003	**0.320 ± 0.001**
	7.05	14.74	23.07	48.71	87.17	**105.12**
Ascorbic acid	0.192 ± 0.003	0.215 ± 0.002	0.236 ± 0.006	0.263 ± 0.01	0.313 ± 0.009	**0.371 ± 0.008**
	23.07	37.82	51.28	68.58	100.64	**137.82**
Control	**0.156 ± 0.004**					

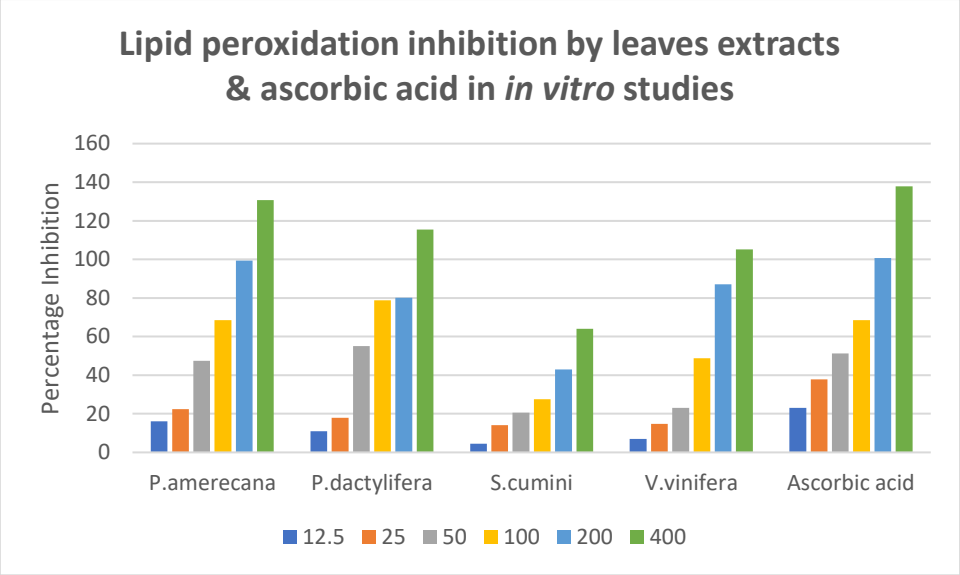

Figure No. 1: Lipid peroxidation inhibition by selected leaves extracts & ascorbic acid in in vitro studies

IV. CONCLUSION

In the present study the *in vitro* antioxidant activity of the selected leaves extracts of *Persea americana, Phoenix dactylifera, Syzygium cumini* and *Vitis vinifera* were evaluated by inhibiting lipid peroxidation in comparison with a known antioxidant ascorbic acid. The selected leaves extracts showed the inhibition of lipid peroxidation in the order of *P.americana* > *P.dactylifera* > *V.vinifera* > *S.cumini*..

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