REPORT

ANTI LIPID PEROXIDATION ACTIVITY OF *PIPER TRIOICUM ROXB.* AND *PHYSALIS MINIMA L.* EXTRACTS

SATHIS KUMAR DINAKARAN[1]*, NARASIMHA RAJU SARASWATHI[1], VENKATA RAMA RAO NALINI[2], SRISUDHARSON[1], VENKAT RAM REDDY BODANAPU[3], HARANI AVASARALA[1] AND DAVID BANJI[1]

[1]Nalanda College of Pharmacy, Nalgonda-508001, Andhra Pradesh, India
[2]Vels College of Pharmacy, Chennai, Tamilnadu, India
[3]KK College of Pharmacy, Chennai, Tamilnadu, India

ABSTRACT

Attempt has been made to evaluate free radical scavenging activity of ethanolic extract of *Piper trioicum Roxb.* and *Physalis minima L.* individually. In this study goat liver has been used as lipid source. This *in vitro* evaluation was done by measuring the malondialdehyde (MDA) of tissue homogenates. The results suggest that the ethanolic extract of the *Piper trioicum Roxb.* and *Physalis minima L.* has the ability to suppress the lipid peroxidation and it was also found that *Piper trioicum Roxb.* extract has more activity than *Physalis minima L.* extract.

Keywords: *Piper trioicum Roxb.*, *Physalis minima L.*, Antioxidant, Malondialdehyde.

INTRODUCTION

Pipertrioicum Roxb. belongs to Piparaceae family, distributed in South Asian countries. The whole plant is used as rubefacient, diuretic, hepatoprotective and used for diabetes, muscular pains, headache, toothache and cholera in folk medicine; the root is used as diuretic (Madhava Chetty *et al.,* 2008). *Physalis minima L.* belongs to Solanaceae family, distributed in South Asian countries. It is commonly found on the bunds of the fields, waster lands, around the houses, on roadsides, etc., where the soil is porous and rich in organic matter. It is an annual herbaceous plant having a very delicate stem and leaves. The whole plant is bitter, appetizing, tonic, diuretic, laxative, and useful in inflammations, enlargement of the spleen and abdominal troubles (Warrier, *et al.,* 2006). Researchers isolated a new 13, 14-seco-16, 24-cyclosteroid, physalin L, along with known compounds, from Physalis minima (Sen.*et al.,* 1995). Anti-inflammatory, analgesic and antipyretic activities of Physalis minima Linn, investigated the crude methanol extract and chloroform fraction of the whole plant of *Physalis minima* Linn (Solanaceae) for anti-inflammatory, analgesic and antipyretic activities in NMRI mice and Wistar rats of either sex at 200 and 400 mg/kg, respectively (Murad Ali Khan *et al.,* 2009). Lipid peroxidation is oxidative deterioration of ply unsaturated lipid that occurs through free radical chain reaction (Halliwell and Gutteridge, 1989). Free radicals are generated inside the body and cause several damages to vital cellular organs. To control and reduce lipid peroxidation antioxidants have been proven helpful to a significant extent. As lipid peroxides are increasingly thought to have a pathogenic role in many disorders a specific and reliable test for their concentrations in plasma in greatly needed. The present work has been carried out *in vitro* to evaualte the antioxidant effect of ethanolic extracts of *Piper trioicum Roxb.* and *Physalis minima L.*

MATERIALS AND METHODS

The study has been performed on the goat (*Capra Capra*) liver using common laboratory marker of lipid peroxidation like measurement of the malondialdehyde (MDA) content of tissue. The goat liver was selected because of its easy availability and close similarity to the human liver in its lipid profile.

Plant material

Plant material of *Piper trioicum Roxb.* and *Physalis minima L.* were collected from local areas of Talakona and local areas of Nalgonda, Andhra Pradesh respectively and both were authentified by Mr. Madhavachetty, Botanist, SV University, Thirupati, Andhra Pradesh.

Extraction

Plants were dried in the shade and ground into uniform powder using milling machine (Evans, 2007). The extraction procedures were carried out for about 18 hrs using soxhlet apparatus with 70% ethanol as a solvent. Initially the shade dried plant of *Piper trioicum Roxb.* and *Physalis minima L.* was taken individually in a grinder mixture to obtain a coarse powder and then passed through a 40 mesh (Kokate *et al.,* 2004). The powder

**Corresponding author:* e-mail: satmpdina@yahoo.co.in

(500gms) of both plants was defatted with hexane and later extraction procedure was carried out using ethanol. Then residue was collected separately and used for experiment. The two extracts, *Piper trioicum Roxb.* and *Physalis minima L.* was concentrated to dryness under reduced pressure separately. The ethanolic fractions of both plants were subjected to qualitative chemical investigation for identification of Phytoconstituents (Khandelwal, 2005). The data are presented in table 1.

Table 1: Phytochemical investigation

S. No.	Test	*Physalis minima* L.	*Piper trioicum* Roxb.
1	Carbohydrate Molish test	+	+
2	Proteins Biuret test	-	-
3	Amino acid Nin-hydrine test	-	-
4	Steroids Lieberman burchand test	-	-
5	glycosides Killerkilliani test	+	+
6	Test for alkaloids Dragendroffs test	+	+
	Mayer's test	+	+
	Hager's test	+	+
	Wagner's test	+	+
7	Test for Tannins and phenolic compounds Lead acetate Solutions: Acetic acid Solution 5% $FeCl_3$ Solution.	+	+
8	Test for flavonoids Shinado test	-	-
9	Test for Saponins	-	-
10	Test for Terponoids	-	-

Note: + = presence, - =Absence

Animal material

Goat liver was collected from local market of Nalgonda. Goat liver perfused with normal saline through hepatic portal vein was harvested and its lobes were briefly dried between filter papers to remove excess blood and thin cut with a heavy-duty blade. The small pieces were than transferred in a sterile vessel containing phosphate buffer (pH 7.4) solution. After draining the buffer solution as completely as possible, the liver was immediately grinded to make a tissue homogenate (1g/ml) using freshly prepared phosphate buffer (pH 7.4).

Antilipid peroxidation activity

Ethanolic extracts of *Piper trioicum Roxb.* and *Physalis minima L.* were used in various concentrations (2000, 1500, 1000 and 500 µg/ml) individually. 3ml of liver homogenate was added with 100 µl of 15mM ferric chloride solution and was shaken for 30 min. From collected mixture, 100 µl was added with 1ml of different concentration of both plant extracts individually in different test tubes. The same procedure was followed for control and blank. Water was used as a control and ascorbic acid (1000µg/ml) as standard. All the test tubes were incubated for 4hrs at 37°C. After incubation, Trichloroacetic acid (TCA) was added to all tubes containing the mixture in 1:1 ratio and centrifuged for 30 min. The supernated liquid was collected and thiobarbituric acid (TBA) was added in 1:1 ratio and heated for 1 hr in water bath, cooled and absorbance was measured at 530nm. By using the following formula the percentage of anti lipid peroxidation activity was calculated.

$$\text{Percentage of anti lipid peroxidation} = \frac{(\text{Control} - \text{Sample})}{(\text{Control})} \times 100$$

Statistical analysis

All data was subjected to analysis of variance (ANOVA). The data (mean±standard deviation) shown are mean value and the significance differences was compared by using Dennett's Multiple comparison test at the $p<0.05$ probability level. ANOVA was carried out by using GRACHPADPRISM version 4.2 software.

Table 2: Antilipid peroxidation activity of *Piper trioicum* Roxb. and *Physalis minima* L.

Concentration (µl/ml)	% inhibition activity		
	Piper trioicum Roxb.	*Physalis minima* L.	Standard
500	23.11±1.871**	43.16±1.472**	
1000	25.24±2.551**	71.23±1.080**	68.16±1.780**
1500	36.79±1.080**	70.75±0.707**	
2000	33.96±1.080**	48.35±1.080**	

The values are mean ± SD of 6 values. Means with superscripts (**) within a column are significantly different from each other at *p<0.01*as determined by Dennett's Multiple comparison test. F value is 534.9, df (5, 12).

RESULTS

The ethanolic extract of *Piper trioicum Roxb.* and *Physalis minima L.* were concentrated on water bath to a dry residue and kept in a desiccator. The percentage yield was 14.3%w/w and 5.8%w/w for ethanolic extract of *Piper trioicum Roxb.* and *Physalis minima L.* respectively.

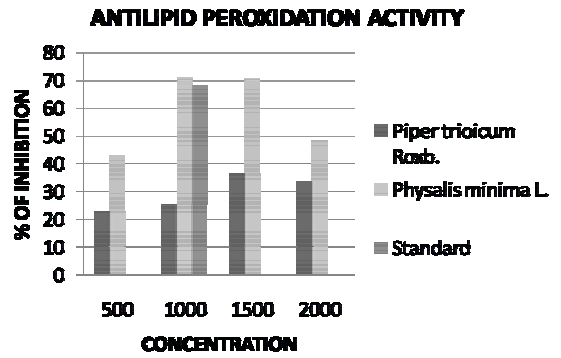

Fig.1: Anti-lipid peroxidation activity test.

The Phyto chemical screening and quantitative estimation of the percentage crude yields of extracts studied has shown that the whole plants of *Piper trioicum Roxb.* and *Physalis minima L.* were rich in alkaloids, carbohydrates, phenolic compounds and tannins. In this study, the experiment was performed to analyze the anti-hyperlipidemic effect of ethanolic extract of our plants on goat liver homogenate from 500mcg/ml to 2000mcg/ml by measuring the levels of malondialdehyde (MDA) which is produced based on the acid-catalyzed decomposition of lipid peroxides. The analysis of data confirms that anti lipid peroxidation activity was maximum at 1500mcg/ml of *Piper trioicum Roxb.* and at 1000mcg/ml and 1500mcg/ml of *Physalis minima L.* compared to 1000mcg/ml of standard ascorbic acid. In case of *Piper trioicum Roxb.*, antioxidant activity was gradually increased up to 1500mcg/ml then it decreased at 2000mcg/ml. But in case of *Physalis minima L.* anti lipid peroxidation activity was increased up to 1500mcg/ml, then its anti oxidant activity was decreased at 2000mcg/ml. From the experimental results, it was certainly confirmed that the ethanolic extracts of both plants have potent antioxidant property and also that the ethanolic extract of *Piper trioicum Roxb.* has potent antioxidant property in comparison to ethanolic extract of *Physalis minima L.*. The data are presented in table 2 and fig.1.

DISCUSSION

Oxidation stress has been implicated in the pathology of many diseases and conditions including cardiovascular disease, diabetes, inflammatory conditions, cancer and ageing. Anti-oxidants may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid per-oxidation and other mechanisms (Nayak, *et al.*, 2005). The ethanolic extracts of both plants are able to reduce thiobarbituric acid reactive substances (TBARS) significantly when compared with a standard anti oxidant ascorbic acid. The ethanolic extracts of these plants is found to inhibit lipid peroxidation significantly in goat liver homogenate which indicate the strong free radical scavenging and anti lipid peroxidation properties. In conclusion the present study demonstrates that *Piper trioicum Roxb.* and *Physalis minima L.* has significant antilipid peroxidative and free radical scavenging activity which might be helpful in preventing of suppressing the progress of various oxidative stress related diseases.

ACKNOWLEDGEMENT

We are thankful to the management, Nalanda College of Pharmacy, Nalgonda, Andhra Pradesh for providing the support for the successful completion of this work.

REFERENCES

Evans WC (2007). Treas and Evans Pharmacognosy, Elsevier, New Delhi, India, 15th Reprint Edition, p. 64.

Halliwell B, Gutteridge JM (1989). Free radicals in biology and medicine, 2nd edition, Oxford University Press, Oxford.

Khandelwal K (2005). Practical Pharmacognosy, Nirali prakashan, Pune, India. p.149.

Kokate CK, Purohit AP and Gokhale SB (2004). Pharmacognosy, Nirali Prakashan, Pune, India, 29th Edition, p.54.

Madhava Chetty K, Sivaji K and Tulasi RK (2008). Flowering plants of chithor district, Andhra Pradesh, India, Students offset printers, Tirupati, first edition, p.301.

Murad Ali Khan, Haroon Khan, Sarwar Khan, Tahira Mahmood, Pir Mohammad Khan and Abdul Jabar (2009). Anti-inflammatory, analgesic and antipyretic activities of Physalis minima Linn, *Journal of Enzyme Inhibition and Medicinal Chemistry*, **24**(3): 632-637.

Nayak UK, Mariappan G, Mohanty JP, Bhuyan NR and Patro BK (2005). Antilipid peroxidation activity of *Curcuma aromatica* Linn, *Indian Drugs*, **44**(6): 483-485.

Sen G and Pathak HD (1995). Physalin L, a 13,14-seco-16,24 cyclosteroid from Physalis minima, *Phytochemistry*, **39**(5): 1245-1246.

Warrier PK, Nambiar VPK and Ramankutty C (2006). Indian Medicinal plants, compendium of 500 species, Orient Longman private limited, Chennai, India, Vol.4, reprinted.