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**Study of the antioxidant activity of a traditional herbal oil used for cancer therapy in Sri Lanka.**

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### **Abstract**

In Asian countries like Sri Lanka and India, there are well-developed traditional medical systems, which use various herbal preparations to treat cancer. These herbal formulations are prepared using a mixture of plant materials according to traditional formulas. The therapeutic benefit of these medicinal preparations is often attributed to their antioxidant properties. Many antioxidant compounds, naturally occurring in plant sources have been identified as free radicals or active oxygen scavengers those of which play a significant role in prevention and cure of cancer through elimination of carcinogens from the system. It has been shown that phenolic substances present in plants have major contribution for their antioxidant activity.

The objective of this study was to investigate the antioxidant activity of a traditional herbal oil which is called 'pranajeewa' which is used in certain types of cancer and other diseases in traditional medicine of Sri Lanka. Antioxidant activity of the oil was investigated by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method, reducing power and anti lipid peroxidation activity (TBARS assay). These values were compared with ascorbic acid and vitamin E. The phenol content was

investigated by Folin-Ciocalteu method. The polyphenol content of the oil was  $0.29 \pm 0.09$  % w/w gallic acid equivalents. The  $EC_{50}$  value for DPPH radical scavenging activity of the oil was  $79.84 \pm 5.35$   $\mu$ l/ml. The  $EC_{50}$  value for TBARS assay of the oil was  $19.24 \pm 0.52$   $\mu$ l/ml.

The above findings indicate that 'Pranajeewa' oil has free radical scavenging activity and antilipid peroxidation activity.

### **Introduction**

Since ancient times herbal medicines have been used by traditional medical practitioners for the treatment of cancer. Many of these herbal formulations are prepared using a collection of plant materials according to traditional formulas. Although these herbal medicines have been prescribed to patients for so many years, most of them may have not been subjected to scientific investigation to determine whether these formulations truly have the potential to be benefit of the patients.

The therapeutic activity of herbal medicinal compounds towards cancer is often attributed to their antioxidant properties. The present study was carried out to investigate the antioxidant activity of traditional herbal oil called 'Pranajeewa'. The pranajeewa oil, which is very bitter, is a mixture of five varieties of oils and over 200 kinds of herbs found in the forests of Sri Lanka, India and other herb producing countries.

Antioxidant activity was evaluated by DPPH radical scavenging activity, reducing power and antilipid peroxidation activity (TBARS assay).



Figure 1.1: The “Pranajeewa” herbal oil

## Methodology

### DPPH radical scavenging activity

Free radical scavenging activity was assayed by DPPH method [Blois MS. (1958)]

Different concentrations of drugs were prepared (7.5, 5.0, 4.0, 3.0, 2.0, 1.0, 0.5, 0.25  $\mu\text{l dm}^{-3}$ ) in ethanol solution.

- A volume of 50  $\mu\text{l}$  of the drug sample was mixed with 450  $\mu\text{l}$  of DPPH(450  $\mu\text{g dm}^{-3}$ ).
- The mixture was allowed to stand for 30 minutes in dark at room temperature.
- Deionized water (50  $\mu\text{l}$ ) was used as the control.
- The absorbance was measured at 517 nm.
- Ascorbic acid was used as the standard antioxidant.
- % inhibition =  $(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}) / \text{Absorbance}_{\text{control}} * 100$

- The percentage inhibition curve was plotted against the concentration and EC<sub>50</sub> values (concentration of herbal oil needed to inhibit 50 % the radical) were obtained.

### **Determination of antilipid peroxidation activity using TBARS assay.**

Thiobarbituric acid reactive species (TBARS) assay was used to measure the potential anti lipid peroxidation activity of the herbal oil using egg yolk as lipid rich media (*Perera et al.* 2008).

- A volume of 100 µl of egg yolk(10% w/v) in KCl (1.15%) and 50 µl of sample prepared in ethanol (5,10,12,14,16,20,30 µl dm<sup>-3</sup>) were added to 300 µl of 20% acetic acid (pH 3.5).
- This was followed by 300 µl of thiobarbituric acid [0.8% (w/v)] in sodium dodecyl sulphate(1.1%).
- The resulting mixture was vortexed and then heated to 95 °C for 60 minutes in a heat block.
- After cooling to room temperature, a volume of 750 µl of butan-1-ol was added to each tube, vortexed and centrifuged at 3000 rpm for 10 minutes.
- The absorbance of the organic layer was measured at 532 nm.
- Same amount of deionized water was used as the control.
- Vitamin E was used as the positive control.
- The absorbance measured was converted to the percentage anti- oxidant index.
  
- % anti oxidant index =  $(1-T)/C*100$

C: Absorbance value of fully oxidized control

T: Absorbance value of the test sample.

### **Measurement of reducing power**

The reducing power of the oil was determined by the method of *Dhalwal et al.* (1986) with slight modifications.

- Different concentrations (1000, 500, 250, 125, 62.5, 31.25  $\mu\text{L dm}^{-3}$ ) of the herbal oil (100 $\mu\text{L}$ ) was mixed with 250  $\mu\text{L}$  of phosphate buffer (pH6.6) and 250  $\mu\text{L}$  of potassium ferricyanide (1%).
- The mixture was incubated at 50  $^{\circ}\text{C}$  for 20 minutes and 250  $\mu\text{L}$  of trichloroacetic acid (1%) was added.
- The resultant mixture was centrifuged at 5000 g for 10 minutes.
- The supernatant was mixed with distilled water and  $\text{FeCl}_3$  (0.1%) at a ratio of 1:1:2 and the absorbance were recorded at 700 nm.

#### **Determination of the total phenol content**

Total phenol content was determined using the Folin-Ciocalteu method.

- A volume of 50  $\mu\text{L}$  of the water extracts of each drug was diluted with 450  $\mu\text{L}$  of distilled water and Folin-Ciocalteu reagent (1N).
- The mixture was allowed to stand at room temperature for two minutes and 1.25 ml of sodium carbonate (10%) was added.
- Absorbance was measured at 760 nm after 45 minutes.
- Gallic acid was used as a standard in the determination of phenol contents of phenolic compounds were expressed as w/w % gallic acid equivalents.

#### **Data analysis**

All experiments were performed at least three times and data are expressed in Mean $\pm$  Standard deviation (M $\pm$ SD). Linear regression analysis was applied to compute  $\text{EC}_{50}$  using the corresponding dose response curves.

#### **Results and discussion**

Table 1-Total phenolic content, DPPH radical scavenging activity and anti lipid peroxidation activity of the tested oil.

	Total phenol content (% w/w gallic acid equivalents)	DPPH radical scavenging activity EC <sub>50</sub> (μl dm <sup>-3</sup> )	Antilipid peroxidation activity (TBARS assay) EC <sub>50</sub> (μl dm <sup>-3</sup> )
Oil	0.29 ± 0.09%	79.84 ± 5.35	19.24 ± 0.52
Ascorbic acid		3.40	
Vitamin E			12.56± 0.37

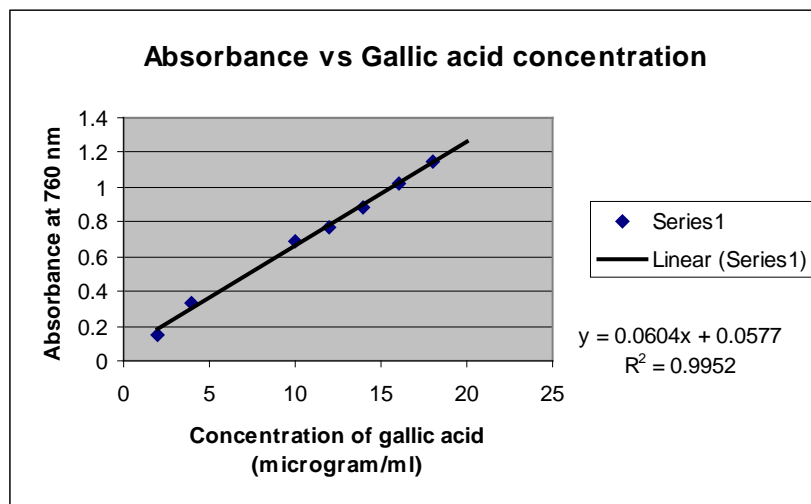


Figure1: Calibration curve for gallic acid at different concentrations (n=3)

Table2: % w/w gallic acid equivalent for the oil at different experiments

Average absorbance	% w/w gallic acid equivalent
0.829	0.30
0.639	0.28
0.215	0.36

Average % w/w gallic acid equivalent =  $0.29 \pm 0.09$  %

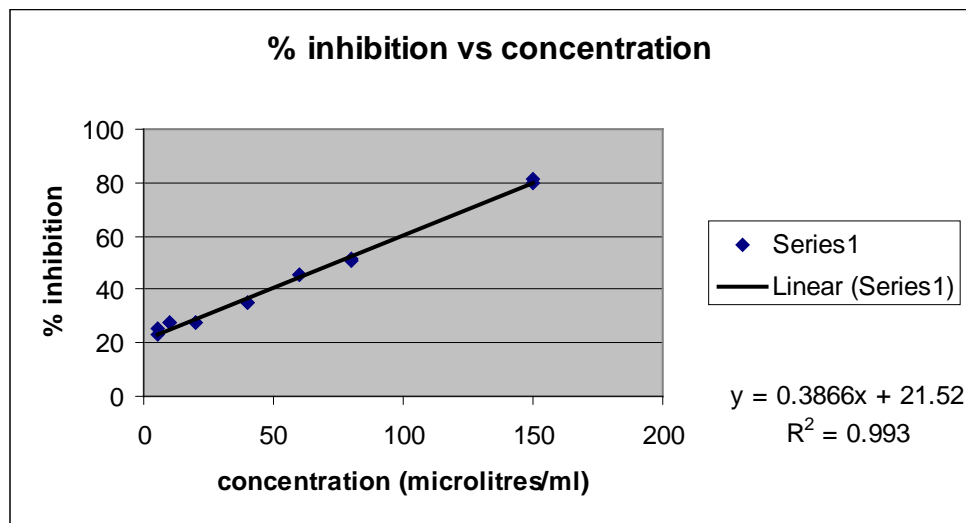


Figure2: Free radical scavenging activity of the “Pranajeewa oil” at different concentrations (n=3).

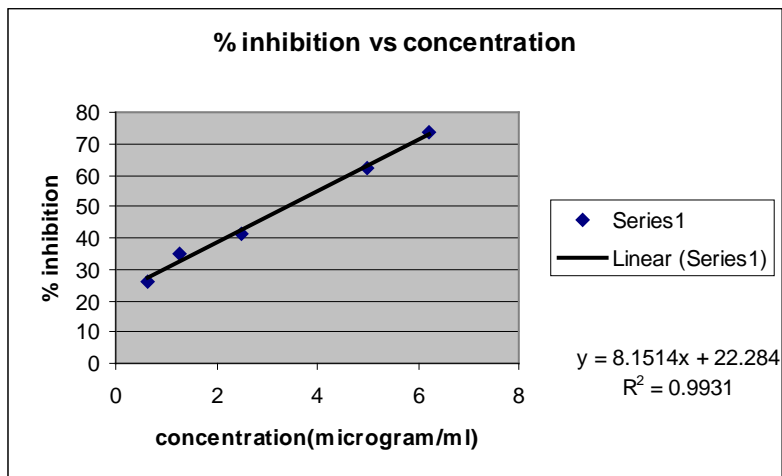


Figure3: Free radical scavenging activity of the L-ascorbic acid at different concentrations(n=3).

Table 3: The DPPH radical scavenging activity of the oil at different concentrations

Experiment	DPPH radical scavenging activity ( EC <sub>50</sub> ) (µl/ml)
1	83.18
2	82.67
3	73.67
Average EC <sub>50</sub>	79.84 ± 5.35
Ascorbic acid	3.40 µg/ml

Table 4: The anti lipid peroxidation activity of the oil at different experiments with compared to vitamin E on TBARS assay

Experiment	Anti lipid peroxidation activity activity ( EC <sub>50</sub> ) (µl/ml)
1	18.97
2	18.91
3	19.94
Average	19.24 ± 0.52
Vitamin E	12.56 ± 0.37

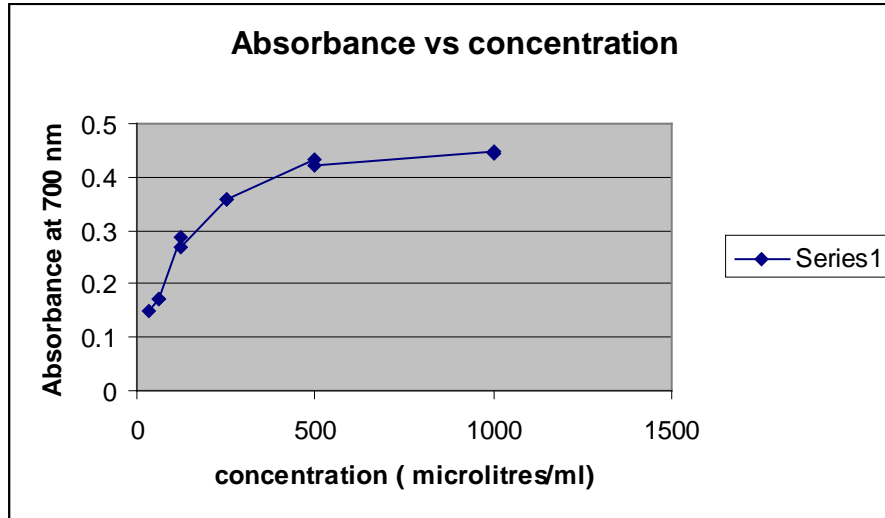


Figure 4: Reducing power of the “Pranajeewa oil” at different concentrations(n=3)

## References

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