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IN-VITRO PHYTOCHEMICAL SCREENING OF *ECLIPTA PROSTRATA* LEAVES EXTRACTS

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Abstract

Eclipta prostrata (L.) is an annual herb belong to family Asteraceae and widely distributed. This plant has been exploited as traditional or folk medicinal plant in various traditional medicine systems in India and other part of world also. *E. prostrata* were extracted with different solvents *i.e.* petroleum ether, chloroform, aqueous, methanol by maceration. These extracts were further investigated for their antioxidant properties in relation to their phytochemical constituents. Methanolic extract showed more maximum antioxidant activity as compared to other extracts. Maximum total phenolic and flavonoid contents was also higher in methanolic extract followed by the aqueous and chloroform extract. As methanolic extract showed significant TPC, TFC and antioxidant, this extract exploited as an effective and safe antioxidant source, on a commercial basis for the development of new phytodrugs.

***Key words*: *Eclipta prostrata*, Total Phenolic Content, Total Flavonoid Content, Antioxidant Activities**

Introduction

Human beings have always been depended upon nature and natural products for all their requirements; plants being the key foundation for medicines, shelter, food, clothing, *etc*. The familiarity about the nutritive and medicinal properties of plants has been passed within and among different civilizations. As a result, plants are significant member of ethno- pharmacology and directly used for treating numerous health ailments by majority of cultures around the world. Plant based products are of great interest due to being cost effective, with lesser after effects and multifaceted applications (Chopra *et al.,* 1956; Joshi and Joshi, 2000; Jimoh *et al.,* 2011). Even today, plants are not only indispensable in health care but are also the best source for safe future drugs.

Natural products are backbone of traditional system of medication globally and they have been an integral part of history and culture. Traditional Indian system of medicine known as Ayurveda (mother of all therapies) is considered as the oldest health care system on the planet earth. It is still widely practiced not only in India but also in other Asian other countries (Chatterjee, 2001).

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Eclipta prostrata (L.) is an annual herbaceous plant, belonging to the Asteraceae family. Morphologically it is prostrate or erect stem; branches are present, hairs are present; the leaves are sessile, opposite and lanceolate (Kirtikar and Basu, 1999). Mostly this plant is known in the three major forms of traditional medicinal systems in the India *i.e* Ayurveda, Unani, and Siddha, as bhringoraaja, bhangraa, and karissalaankanni, respectively. In Ayurveda this plant considered as hepatoprotective herb (Mehra and Handa, 1968). *Eclipta* is used in traditional medicine for infectious diseases. It is believed that this medicinal herb possesses anti- aging and rejuvenate teeth, bone, sight, and hearing activity. *Eclipta* has been studied for its pharmacological and insecticidal activities and antioxidant, antihepatotoxic, anticancer immunomodulatory, anti-inflammatory and antidiabetic activities (Erdo Urul, 2002). The current investigation was planned to investigate the plant extracts of *E. prostrata* for their phytocompounds and antioxidant properties.

Material and methods

Preparation of Plant Extracts

The leaves and stem of *Eclipta prostrata* were collected from district Patiala, Punjab (India). After

washing with running tap water and drying at room temperature, the collected plant material was grounded into coarse powdered. 50 gm of leaf powder was extracted by maceration in four different solvents (250 mL,) *i.e.* methanol, aqueous, chloroform and petroleum ether. After 72 hours leaf extracts were filtered through whatman filter paper and solvent was evaporated by using a rotary evaporator. The extracts were stored in airtight glass containers and stored at 4°C till further use.

Estimation of Total Phenolic Content (TPC)

Total Phenolic Content was determined as per the method of (Singleton and Rossi, 1965) with minute changes. 1mL of Plant extract was taken in a test tube and add 5mL of folin ciocalteu reagent and 2 mL of Na_2CO_3. The solution was vortexed and incubated for 15 minutes in dark. Absorbance was measured at 620 nm. Gallic acid was used as standard. Phenolic content was calculated from calibration curve by using excel ($Y = 0.0164x+0.0557$, $R^2 = 0.9964$). All tests were performed in triplicates. The amount of TPC was determined as per the formula given below and it was expressed as mg/g of dry weight equivalent to Gallic acid (mg GAE/g):

$$T = C \times \frac{V}{M}$$

T = TPC; C = Gallic acid concentration determined from calibration curve (mg); V = PE volume (mL); M = PE weight (gm)

Estimation of Total Flavonoid Content (TFC)

Total flavonoid Content was estimated by Aluminium Chloride Colorimetric assay (Aiyegroro and Okoh, 2010). 1mL of Plant extract was taken, add 3 mL of methanol, 0.2 mL of 10% aluminium chloride, 0.2 mL of 1M potassium acetate and distilled water (5.6 mL). The mixture was vortexed and left at room temperature for 30 minutes and absorbance was measured at 420 nm. Quercetin was used as reference or standard. All tests were performed in triplicates. Total flavonoid content was estimated from the calibration curve using the equation ($Y=0.0047x+0.0391$, $R^2 = 0.996$). The formula given below was used for the estimation of TFC and it was expressed as mg/gm of Quercetin equivalent (mg QE/g):

$$T = C \times \frac{V}{M}$$

T = TFC; **C** = Quercetin concentration estimated from calibration curve (in mg); **V** = Plant Extract volume (mL); M = Weight of plant extract (gm)

Antioxidant Studies

Non-Enzymatic Assays

DPPH assay

Antioxidant potential of all the extracts of five plants was determined by DPPH (1, 1-diphenyl-2-picryl-hydrazyl) free radical scavenging assay (Blois, 1958). 1 mL of plant extract of different concentrations (10 -100 μg/mL) was taken and 1 mL of 0.3mM DPPH was added to it under dark conditions and incubated for 30 minutes. Absorbance was taken at 517 nm by UV-Visible spectrophotometer. Ascorbic acid was used as reference or standard with the same concentrations (10-100 μg/mL). Percentage inhibition was calculated by the formula given below:

$$\% \text{ Inhibition} = \frac{\text{Absorbance control-Absorbance sample}}{\text{Absorbance control}} \times 100$$

ABTS assay

ABTS (2, 2'-azino-bis 3-ethylbenzothiazoline-6-sulphonic acid) assay was performed according to the method of Shirwaikar *et al.,* (2006) with slight modifications. ABTS radical cation (ABTS) was obtained with the help of reaction of 7mM ABTS solution with 2.45 mM ammonium per sulphate. Reaction mixture was kept in dark at room temperature for about 12-16 hours before performing the experiment. ABTS solution was adjusted with ethanol to get an absorbance of at 745 nm. 1 mL of ABTS was added to 1 mL of plant extract with different concentration 10 -100 μg /mL under dark conditions and incubated for 30 minutes in dark. Experiment was performed in triplicates and mean value was recorded. Percentage inhibition was calculated by the formula given below:

$$\% \text{ Inhibition} = \frac{\text{Absorbance control-Absorbance sample}}{\text{Absorbance control}} \times 100$$

Determination of IC-50 value

IC-50 value is defined as the amount of PEs that quench 50% free radicals of DPPH and ABTS. IC-50 value (μg/mL) was determined by plotting graph of percentage inhibition against different concentrations of PEs.

Enzymatic Assay

Preparation of plant extracts

Plant extract was prepared by homogenising plant material with 5 mL of cold extraction buffer containing 100mM potassium phosphate buffer (pH 7.0) and 0.1mM sodium EDTA. Homogenate obtained was filtered

through three layers of muslin cloth and centrifuged at 14000 rpm for 20 minutes at 4°C. The supernatant obtained was directly utilized for all the enzymatic assays.

Catalase assay

Catalase enzyme activity was assessed by the method described by Aebi, (1984). 3 mL mixture was prepared containing 100 μL plant extract, 10 mM H_2O_2, and 50 mM potassium phosphate buffer (pH 7.0) and decrease in absorbance was monitored at 240 nm in every 30 second for 5 minutes. Catalase activity was expressed as µmoles of H_2O_2 decomposed mg-^{1} fresh weight min-^{1}. Experiment was performed in triplicate and mean value was recorded. One unit of catalase is defined as when there is 1.0 μmole of H_2O_2 per min get decomposed pH 7.0 and 25 °C temperature (EC of H_2O_2 is 39.4 mM-^{1}cm-1).

Superoxide dismutase (SOD) assay

SOD assay was performed according to the method of Mishra and Fridovich (1972). 3 mL reaction mixture containing 100 μL enzyme extract, potassium phosphate buffer (50mM), methionine (13mM), NBT (75mM), riboflavin (2μM) and EDTA (0.1mM) was prepared in a test tube. After shaking, these test tubes were placed in the light intensity of 5000 lux. The reaction was allowed to run for 25 minutes. and then it was stopped by switching off the lights. The absorbance was measured at 560 nm. Reaction mixture which didn't produce any colour, served as the negative control. Experiment was performed in triplicates and mean value were recorded. One unit of SOD activity is defined as the enzyme's amount required for the 50% inhibition of the NBT per unit time when measured at 560 nm.

Glutathione s-transferase (GST) assay

GST assay was performed by the method of Habig *et al.,* (1974). 3 mL reaction mixture containing 100μL enzyme extract, 97mM potassium phosphate buffer (pH-6.5), 1mM EDTA, 30mM CDNB and GSH 75Mm was prepared. Absorbance was recorded at 340 nm for every 30 second till 5 minutes. Experiment was performed in triplicate and mean value was recorded. One unit of GST enzyme activity is the enzyme's amount which produces 1.0 µmol of GS-DNB conjugate per min under the experimental conditions (EC of GS-DNB conjugate at 340 nm is 9.6 $mM^{-1}cm^{-1}$).

Results and Discussion

Percentage Yield

Percentage yield of all the plant extracts was determined and the results obtained are shown in Table 6. Maximum yield was noticed with the methanol followed by acetone. Least was obtained in the Petroleum ether. Table 1 showed percentage yield of the plant extracts prepared in different solvents.

Table 1: Yield of leaf extracts of *Eclipta prostrata* in different solvents

Sr. No.	Extract	Method of extraction	Yield (%) W/V
1	Methanol	Maceration	6.8
2	Aqueous		3.6
3	Chloroform		2.5
4	Petroleum ether		1.8

Total Phenolic Content

All the four solvent extracts were investigated for the presence of Total Phenolic Content. Gallic acid standard curve was prepared and is shown in Fig. 1. Maximum phenolic content was found in methanolic extract (69.13±0.72) followed by aqueous extract shown in Table 2 and Fig. 2. The least value of phenolic content (41.59± 0.19) was observed in case of petroleum ether extract.

Total Flavonoid Content (TFC)

The result of total flavonoids contents (TFC) of the four crude extracts of is given in Table 2 and Fig. 3. The TFC in the different crude extracts varied from 9.21 to 24.3 mg quercetin/g dried plant sample. Among the four crude extracts, methanol extract contained the highest

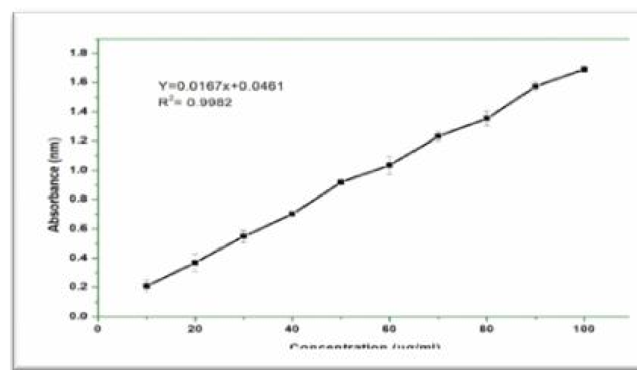

Fig. 1: Standard curve of gallic acid

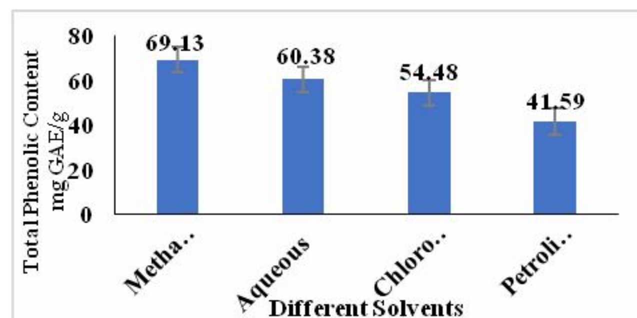

Fig. 2: TPC of plant extracts prepared in different solvents

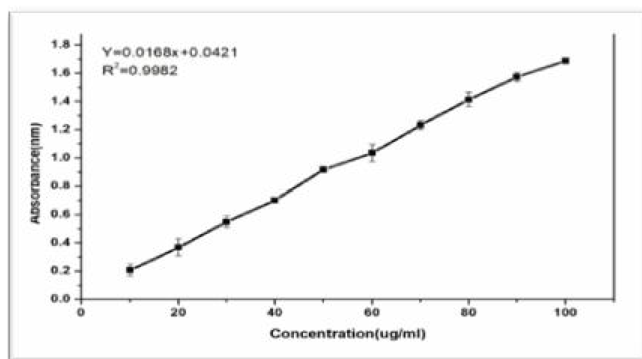

Fig. 3: Standard curve of Quercetin

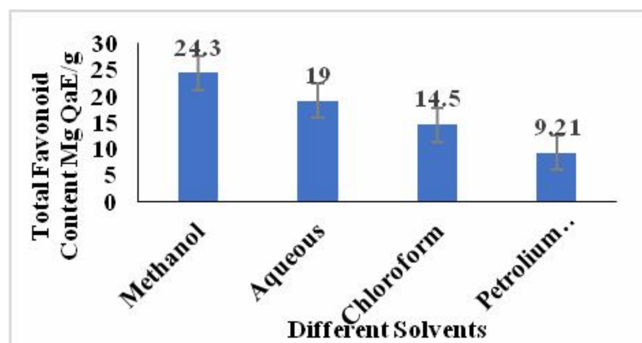

Fig. 4: TFC of plant extracts prepared in different solvents

Table 2: Total phenolic and flavonoid content in leaves of *E. prostrata*

Solvents	Total Phenolic content (mg/g)	Total Flavanoids content (mg/g)
Methanol	69.13±0.722	24.13±1.02
Aqueous	60.38±0.632	19±0.57
Chloroform	54.48±0.432	14.5±0.18
Petroleum ether	41.59±0.19	9.21±0.44

Values are the average of triplicate experiments and values expressed as mean ± SEM

(24.30 mg/g) amount of flavonoids content compounds followed by aqueous (19.0 mg/g), aqueous (14.50 mg/g) and petroleum ether (9.21 mg/g) shown in Fig. 4.

Phytochemical screening is one of the important criteria in identifying and characterisation of new pharmaceutical compounds having medicinal values. Phenolic secondary metabolites are one the most ubiquitous and major group of plant metabolites. Over the last few years phenolics have gained a great attention due to its antimicrobial, antioxidant, antiaging, cytotoxic and anti-inflammatory activities. Flavanoids also active hydroxylated polyphenolic compounds and also possess beneficial effect on health like curing kidney, stomach problem and anti-inflammatory activity.

Non enzymatic assays

Antioxidant potential of the plant extracts was determined by DPPH and ABTS assay and is presented graphically in Figs. 5 and 6. Percentage scavenging activity was estimated for different concentration of plant extracts (10-100µg/mL). Percentage scavenging activity was found to increase with increase in concentration of plant extracts. Ascorbic acid was used as the standard in both the assays. Lower IC-50 value represents high antioxidant activity and *vice -versa*. The highest antioxidant activity was obtained with the methanolic extract in both the assays and percentage scavenging activity varied from 11.86% to 79.03% for DPPH and 25.19 % to 57.20 % for ABTS, respectively, for different concentrations. Chloroform assays also showed good free radical scavenging activity. Least antioxidant potential was obtained with the petroleum ether extract. Strong antioxidant activity of methanolic extract might be due to the presence of high amount of phenolic and flavonoid compounds. The scavenging activity of the phenols is mainly, because of the presence of hydroxyl groups in it. Methanolic extract of is found more potent as compared to the other extracts and found similar with some previous reports reported by (Khandelwal, 2008; Kokate, 1999; Edeoga *et al.,* 2009). On a similar pattern our results also showed that highest TPC and TFC are present in methanolic extract.

On a similar pattern of antioxidant potential was also least in case of petroleum ether.

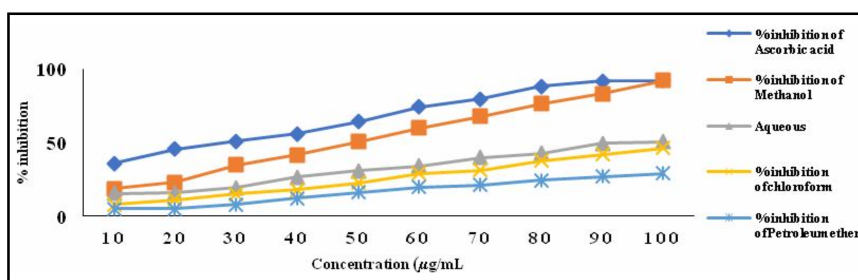

Fig. 5: Percentage inhibition of DPPH radical by different plant extracts of *E. prostrata*

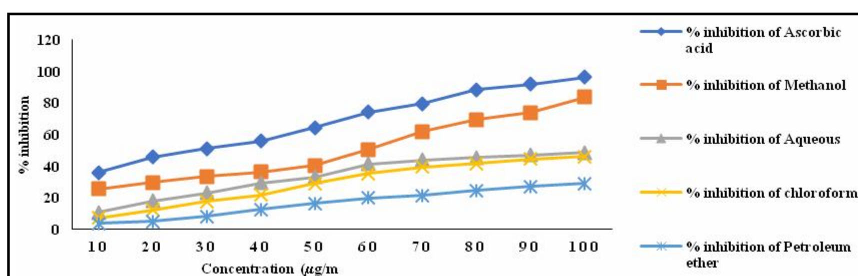

Fig. 6: Percentage inhibition of ABTS radical by plant extracts of *E. prostrata*

IC-50 VALUES

IC-50 values were calculated for all the four extract and are represented in the Figs. 7 and 8. Methanolic extract showed the least value of IC-50 (57.93μg/mL, DPPH) (55.91μg/mL, ABTS) in both the assays, hence exhibiting highest antioxidant activity. Least antioxidant activity was shown by petroleum ether extract as it possessed highest IC-50 value of 139.05μg/mL and 143.96μg/mL value with DPPH and ABTS, respectively presented in Fig. 7 and 8.

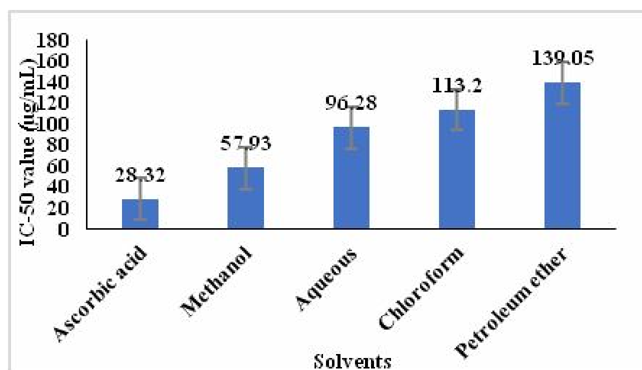

Fig. 7: IC-50 values of plant extract of *E. prostrata* (DPPH)

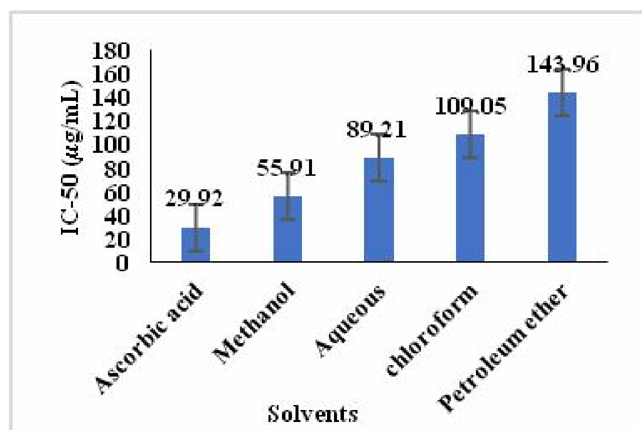

Fig. 8-: IC-50 values of plant extract of *E. prostrata* (ABTS)

Enzymatic antioxidant assay

The antioxidant activity results obtained for antioxidant enzymes namely Catalase, Gluthione S-Transferase and Superoxide Dismutase are shown in table 3. The enzyme activity was found to be 15.13±0.22μmole of H_2O_2 per minute fresh weight for Catalase, 20.35±0.18 µmol of GS-DNB conjugate/min Fresh Weight for Glutathione-S- Transferase and 58.64±0.51 SOD (Unit) Fresh Weight for Superoxide dismutase respectively.

Units of enzyme activities were expressed as:

CAT- One unit of catalase will decompose 1.0 μmole of H_2O_2 per minute at pH 7.0 at 25°C under the assay condition.

Table 3: Antioxidant enzyme activities of *E. prostrata*

Antioxidant enzymes	Activity
Catalase	15.13±0.22
Glutathione-S- Transferase	20.35±0.18
Superoxide dismutase	58.64±0.51

Values are expressed as mean (n=3) ±SD.

SOD- One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of NBT per unit time as monitored at 560 nm.

GST- One unit of GST activity is the amount of enzyme which produces 1.0µmol of GS-DNB conjugate/ min under the conditions of the assay.

Conclusion

Outcomes of current research suggested that this study have significant value especially of methanolic extract with respect to its antioxidant activities. Antioxidant activities may be because of the presence of high amount of phenolic and flavonoid phytoconstituents in methanolic extract followed by aqueous extract. Present study recommended that the methanolic extract can be used as an active and safe antioxidant source, as an ethnomedicine and on a commercial basis for the expansion of drugs. Further, some bioactive elements from the plant should be isolated and assessed for their medicinal properties.

References

Aebi, H. (1984). Catalase *in-vitro*. In Methods in enzymology. *Academic Press,* **105**: 121-126.

Aiyegroro, O.A. and A.I. Okoh (2010). Preliminary phytochemical screening and *in-vitro* antioxidant activities of aqueous extract of *Helichrysum longifolium* DC. *BMC Compl and Alt. Med*, **10**: 21.

Blois, M.S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, **181**: 1199-1200.

Chatterjee, S. K. (2001). Cultivation of Medicinal Plants in India. *World Conference on Medicinal & Aromatic Plants*, Budapest, Germany.

Chopra, R.N., S. L. Nayar and I.C. Chopra (1956). Glossary of Indian Medicinal Plants, CSIR, New Delhi: 4-5.

Edeoga, H.O., D.E. Okwu and B.O. Mbaebie (2005). Phytochemical constituents of some Nigerian medicinal plants. *Afr. J. Biotechnol*, **4**: 685-688.

Erdo Urul, O.T (2002). Antibacterial activities of some plant extracts used in folk medicine. *Pharmaceut. Biol*., **40**: 269-273.

Habig, W.H., M.J. Pabst and W.B. Jakoby (1974). Glutathione S-transferases the first enzymatic step in mercapturic acid formation. *Journal of biological Chemistry,* **249**: 7130-7139.

Jimoh, F.O. and A. A. A. A. J. Afolayan (2011). Comparison of the nutritive value, antioxidant and antibacterial activities of *Sonchus asper* and *Sonchus oleraceus*. *Records of Natural Products*, **5**: 29.

Joshi, A. R. and K. Joshi (2000). Indigenous knowledge and uses of medicinal plants.

Khandelwal, K.R. (2008). Practical Pharmacognosy-Techniques and Experiments, 19th Edition, Nirali Prakashan, Pune.

Kirtikar, K.R. and B.D. Basu (1999). Indian Medicinal Plants, Vol. II. Deharadun (India): International book distributor.

Kokate, C.K. (1999). Practical Pharmacognosy. 4th edition, Vallabh Prakashan, Delhi.

Local Communities of the Kali Gandaki Watershed Area. *Nepal Journal of Ethnopharmacology*, **73**: 175-183.

Mehra P.N and S.S. Handa (1968). Pharmacognosy of Bhringaraja antihepato-toxic drug of Indian origin. *Indian J. Pharma*., **30**: 284.

Mishra, H.P. and I. Fridovich (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological chemistry*, **247**: 3170-3175.

Shirwaikar, A., and A. Shirwaikar, K. Rajendran and I.S.R. Punitha (2006). *In-vitro* antioxidant studies on the benzyl tetra isoquinoline alkaloid *Berberine*. *Biological and Pharmaceutical Bulletin*, **29**: 1906-1910.

Singleton, V.L. J.A. and Rossi (1965). Colourimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J Enol. Vitic,* **16**: 144-158.