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EVALUATION OF ANTIOXIDANT ROLE OF *ERYTHRINA VARIEGATA LINN (Var.alba)*

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ABSTRACT

This present study was to investigate antioxidant activity and the present of phytoconstituents of the Stem bark of the plant *Erythrina variegata* Linn (*EV*) var.alba. The EV extract was prepared in 70% methanol (EVME) as well as water (aqueous) (EVAE). The antioxidant activity is investigated by in-vitro methods like reducing power, superoxide radical inhibition and hydroxyl radical scavenging method and Sodium metabisulphate (SMS) were (25µg/ml) used as standard reference . The maximum percentage of inhibition shown in all activities at 100 µg/ml of EVME and EVAE, in the reducing power activity was 88.78 and 72.58% compared to standard SMS 90.67 and 89.72%, Superoxide radical scavenging, 57.58 and 54.15%, standard SMS were 63.50 and 60.10% and Hydroxyl radical scavenging activity in 1hr incubation were 58.81 and 54.70% and standard SMS 65.60 and 58.10% after 4hr of incubation reports 55.12 and 52.52% and SMS were 62.06 and 56.84% respectively. In phytoconstituents analysis the total flavonoids content was found to be 1.98±0.12 and 1.41±0.10 mg/g, total phenolic content 110±0.32 and 94±0.91 mg/g, total antioxidant capacity 1.74±0.39 and 1.21±0.14 mg/g were reported respectively in EVME and EVAE. The total alkaloid content of EV bark was 0.72%. From our study we can concluded that the stem bark of EV var.alba has a potent antioxidant property.

Key words: *Erythrina variegata*, phytoconstituents, free radicals, antioxidant.

INRODUCTION

The free radicals are the main cause for cellular damage in animals **(Kataria and kataria, 2012)**. Active oxygen exist in different forms such as superoxide anion radicals, hydrogen peroxide, hydroxyl radicals and singlet oxygen these forms of oxygen are highly reactive intermediates and have a collective name of reactive oxygen species (ROS) exert oxidative stress towards the cells of human body. The ROS are necessary for cell function in high concentration leads to oxidative stress and to the development of large number of diseases such as arthritis, carcinogenesis, aging, in addition to endogenous source of free radicals in cells are provided by exogenous sources such as ionizing radiation, tobacco smoking, certain pollutants, organic solvents and pesticides **(Tomovic,*et al*,2015)**.

Antioxidants are added as redox system possessing higher oxidative potential than the drug that is designed to protect or as chain inhibitors of radical inducted decomposition. In general the effect of antioxidant is to break up the chain formed during the propagation process by providing a hydrogen atom or an electron to the free radical and receiving the excess energy possessed by the activated molecule **(Rachh *et al*,2009).**

Many plant drugs have been recognized as a good protector in opposition to the free radicals by trigger antioxidant expression. The natural antioxidant from plant source has been viewed as an economical and secure as an antioxidant to treat many diseases.

The plant ***Erythrina Variegata* Linn (*EV*)** var.alba (Family: fabaceae), Synonym: *Erythrina indica* distribute through India deciduous forest as well as cultivated (**Warrier, 1994**). The plant is medium sized quick growing tree approximately 18 m high armed with dark colored prickles bark, smooth shiny papery leaves with trifoliolate, leaflet 10-15 cm long ovate. The bark of the plant *EV* was reported to possess lignans and related flavonoids which are known antioxidants properties (**Khare, 2007**).

Different parts of *EV* have used in traditional medicine as nervine sedative, febrifuge, anti-asthmatic and antiepileptic **(Cui *et al*, 2009**. In some experiments, it has potential effects for treatment of some diseases like convulsion, fever, inflammation, bacterial infection, insomnia, helminthiasis, cough, cuts and wounds **(Kumar *et al*, 2010)**.

There is less scientific evaluation conducted on *EV* stem bark for confirming its role as an antioxidant. Thus, the study was plan to in-vitro antioxidant activity and phytoconstituents present in EVME and EVAE.

MATERIALS AND METHODS

Plant material collection

The plant *Erythrina Variegata* linn var.alba were collected from the surrounding villages of Kalaburagi district and plant was authenticated by Prof. Dr N G Patil Head of the Department of Botany H.K.E Society's VG women's degree college Kalaburagi, and then stem bark was shade dried at room temperature and pulverized.

Preparation of extract

The bark powder was extracted by using 70% methanol in Soxhlet extraction techniques and cold maceration method used for aqueous extraction. The obtained extracts were concentrated under reduced pressure and stored in desiccators until use (**Khandelwal, 1996).**

Preparation of standard stock solution

The SMS 25 µg/ml was used as standard reference for this study. Its stock solution was prepared freshly in the concentration of 1000 µg /ml in methanol. The 25 µg /ml of SMS were prepared from using this stock solution in methanol. **(Rachh *et al*, 2009)**

Preparation of extract stock solution

70% methanolic and Aqueous extract of *EV* were prepared at the concentration of 1000 µg /ml in methanol and distilled water and by using this stock solution the different concentration viz. 5, 10, 25, 50, 100 µg /ml of EVME and EVAE were prepared in methanol and distilled water. **(Rachh *et al,* 2009)**

Antioxidant activity

Reducing power

The EVME and EVAE were mixed in 1 ml of distilled water so as to get 5µg, 10µg, 25µg and 50µg, 100µg concentration. This was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5ml, 1%). The mixture was incubated at 50°C for 20 minutes. A portion (2.5 ml) of tri-chloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance (OD) was measured at 700nm **(Revansiddaya, *et al,* 2011).** Increased absorbance of the reaction

mixture indicates increase in reducing power. The percentage of increase in reducing power was calculated by using the formula.

$$\text{Percentage of scavenging activity} = \frac{\text{Absorbance of control} - \text{absorbance of Sample}}{\text{Absorbance of control.}} \times 100$$

Superoxide anion scavenging activity

About 1ml of nitroblue tetrazolium (NBT) solution (156µm NBT in 100 mM phosphate buffer, pH7.4), 1 ml NADH solution (468µm in 100 mm phosphate buffer, pH 7.4) and 0.1 ml of sample solution of EVME and EVAE in distilled water was mixed. The reaction was started by adding 100µl of Phenazine methosulphate (PMS) solution (60µ PMS in 100 mM phosphate buffer, pH 7.4) to the mixture. The reaction was mixture was incubated at 25°C for 5 minutes, and the absorbance at 560 nm was measured against blank (**Revansiddaya, *et al,* 2011).** Decreased in absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage of inhibition of OD was calculated by using the formula mentioned reducing power activity.

Hydroxyl radical scavenging activity

Hydroxyl radical generation by phenyl hydrazine has been measured by the 2-deoxyribose degradation assay. In 50 mM phosphate buffer (pH 7.4) containing 1mM deoxyribose, 0.2 mM phenyl hydrazine hydrochloride was prepared. 0.6 ml of 1mM deoxyribose and 0.4ml of EVME and EVAE (varying doses 10,20,25,50 &100 µg/ml) and standard SMS 25µg/ml were taken 0.2ml of phosphate buffer was added to make the volume to 1.6 ml. The reaction mixture was incubated for 10min and 0.4ml of 0.2mM phenyl hydrazine hydrochloride was added and incubated for 1hr or 4 hour and 1 ml each of 2.8% trichloroacetic acid and 1 % (w/v) Thiobarbituric acid were added to the reaction mixture and heated for 10 minutes in a boiling water bath. The tubes were cooled and absorbance taken at 532nm by using UV-double beam spectrophotometer (Shimadzu-UV-1601) (**Jain and setty, 2016**). Decrease in absorbance is indicating the increase in the hydroxyl free radical scavenging activity.

Determination of total flavonoid content

The total flavonoid content of *EV* was estimated according to aluminum chloride colorimetric method, 1 ml of EVME and EVAE of different concentration solution were mixed with 3 ml of methanol, 0.2 ml of aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water. It was kept at room temperature for 30 min and the absorbance of the reaction mixture was measured at 415 nm wavelength with UV-VIS spectrophotometer (UV-1280 Shimadzu) against methanol was used as blank. The total content of flavonoid compounds in EVME and EVAE in quercetin equivalents was calculated by the following equation

$$C=(c \times V) / m$$

Where: C = total content of flavonoid mg/gm of EVME and EVAE, in quercetin equivalent, c = the concentration of quercetin established from the calibration curve in mg/ml, V= the volume of extracts in ml, m= the weight of extracts in gm **(Mclaughlin *et al,* 1998).**

Total Phenolic content

Total phenolic content in *EV* was estimated by using the Folin-Ciocalteu's reagent. The reaction mixture was prepared by mixing 0.1mL of methanolic solution of EVME and EVAE (50 mg mL-1 concentrations), 7.9 mL of distilled water, and 0.5mL of the Folin-Ciocalteu's reagent and 1.5 mL of 20% sodium carbonate. After 2 h the absorbance at 750nm was obtained against blank that had been prepared in a similar manner by replacing the EVME and EVAE with distilled water. The total phenolic contents expressed as mg gallic acid equivalents per g dry weight of *EV* was determined using calibration curve of gallic acid standard **(Rachh *et al* 2010).**

Total Antioxidant capacity

The total antioxidant capacity of *EV* was carried out by the phosphomolybdenum method. 0.3 ml of extract and sub-fraction in methanol, ascorbic acid used as standard (5-200 µg /ml) and blank (methanol and water) were combined with 3 ml of reagent mixture separately and incubated at 95 ^{o}c for 90 min. After cooling to room temperature, the absorbance of each sample was measured at 695 nm against the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid and was calculated by the following equation;

$A = (c \times V)/m$

Where, A= total content of antioxidant compounds mg/gm plant extract in ascorbic acid equivalent, c= the concentration of ascorbic acid established from the calibration curve mg/ml, V= the volume of extracts in ml, m= the weight of extracts in gm **(Wang and jiao, 2000).**

Determination of total alkaloid content

The dried stem bark powder of *EV* was taken and powder was moistening with dilute ammonia solution continuously extracted for 18 hrs with 95% alcohol. Alcohol was removed by distillation. The residue was extracted 5 times with 25 ml of 1N HCL until complete extraction of alkaloid is affected. The mixed solution was transferred into a separating funnel and wash with 5 ml of chloroform. Run off chloroform layer make acidic solution alkaline with ammonia and shake with 5, 25 ml portion of chloroform till complete extraction of alkaloid is affected; wash the combined chloroform extract with two portions each of 5 ml water. Filter the chloroform layer in a flask and evaporate to dryness. Add 5 ml of alcohol to the residue and evaporate to dryness. Repeat the process and weigh the residue to constant weigh in vacuum desiccators and calculate the percentage of alkaloids in dried bark powder **(Rachh *et al,* 2009).**

Statistical analysis

Experimental results were mean ± SEM of three parallel measurements. Linear regression analysis was used to calculate the IC_{50} value.

RESULTS AND DISCUSSION

Reducing power activity

The reducing power has demonstrated dose dependant increase in the reducing power activity, the EVME and EVAE at dose 100 μg/ml have demonstrated maximum reducing Power activity, which are 88.78% (0.353 ± 0.013) and 72.58% (0.343 ± 0.021), in respective extracts and standard drug SMS shown in EVME 90.67 % (0.224 ± 0.021) and in EVAE 89.72% (0.206±0.023). The EVME showed higher reducing power and the antioxidant activity of EVME is more compared to EVAE.

Superoxide anion scavenging activity

This activity revels that the super oxide scavenging increased in dose dependent manner. The EVME and EVAE at the concentration of 100 µg/ml shows significant percentage of inhibition, 57.58% (0.259 ± 0.016) and 54.15% (0.256 ± 0.014) respectively where the standard SMS 63.50 (0.295±0.034) & 60.10 % (0.254±0.052).In this activity EVME is reports greater superoxide scavenging properties then the EVAE.

Hydroxyl radical scavenging activity

In this study after 1hr of incubation the dose dependent increase in hydroxyl radical scavenging activity. The maximum activity was reported in 100 µg/ml concentration. The percentage of inhibition of EVME 58.81 (0.199 ± 0.021) and EVAE 54.70% (0.206±0.015) respectively, whereas standard SMS 65.60% (0.116 ±0.013) and 58.10% (0.210±0.011). After 4hr incubation the percentage of inhibition was decreased in EVME 55.12% (0.184±0.017) and EVAE 52.52% (0.200±0.006) which shows good percentage of inhibition even after 4hr of incubation and it shows significant hydroxyl radical scavenging and EVME have showed more hydroxyl radical scavenging activity then the EVAE.

Total flavonoid content

The total flavonoid content of *EV* in 70% methanolic and Aqueous extract was found 1.98±0.12 and 1.41±0.10 (mg/g of quercetin equivalent).

Total Phenolic content

The total phenolic content of *EV* bark extract of 70% methanolic and Aqueous was determined 110±0.32 mg/g and 94±0.91 mg/g expressed as Gallic acid.

Total Antioxidant capacity

The total antioxidant capacity in 70% methanolic and aqueous bark extracts of *EV* was found 1.74±0.39 mg/g and 1.21±0.14 mg/g expressed as standard ascorbic acid.

Total alkaloid content

The total alkaloid content of *EV* bark powder was found 0.72%.

CONCLUSION

From the this study it can be concluded that the 70% methanolic and aqueous bark extract of the plant *Erythrina Variegata* Linn (var.alba) demonstrated with potent antioxidant properties. The EVME shows the greater free radical scavenging then the EVAE in all studied antioxidant activities. This reported antioxidant activity of *Erythrina Variegata* may be due to the presence of rich amount of flavonoid, phenolic compounds. Further studies are required to evaluate the in-vivo screening for antioxidant potential and isolate secondary metabolite from this extract to deliberate its efficacy in various diseases.

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Table.1

Reducing power scavenging activity of EVME and EVAE

Groups & concentration in µg/ml	Percentage of inhibition EVME*	Percentage of inhibition EVAE*
Control (Reaction mixture)	---	---
Control + Std (SMS) 25 µg/ml	90.67	89.72
Control + Test sample 5 µg/ml	7.57	5.59
Control + Test sample 10 µg/ml	23.89	21.35
Control + Test sample 25 µg/ml	40.25	30.55
Control + Test sample 50 µg/ml	62.37	42.23
Control + Test sample 100 µg/ml	88.78	72.58

*Each value represents mean±SEM (n=3)

Table.2

Superoxide anion radical scavenging activity of EVME and EVAE

Groups & Concentration in µg/ml	Percentage of inhibition EVME* After 1hr	Percentage of inhibition EVME* After 4 hr	Percentage of inhibition EVAE* After 1 hr	Percentage of inhibition EVAE* After 4hr
Control (Reaction mixture only)	---	---	---	---
Control + Std (SMS) 25 µg/ml	65.60	62.06	58.10	56.84
Control + Test sample 5 µg/ml	5.28	4.88	7.29	4.31

Control + Test sample 10 µg/ml	20.74	15.20	14.39	9.75

Groups & Concentration in µg/ml	Percentage of inhibition EVME* After 1hr	Percentage of inhibition EVME* After 4 hr	Percentage of inhibition EVAE* After 1 hr	Percentage of inhibition EVAE* After 4hr
Control (Reaction mixture only)	–	–	--	–
Control + Std (SMS) 25 µg/ml	65.60	62.06	58.10	56.84
Control + Test sample 5 µg/ml	5.28	4.88	7.29	4.31
Control + Test sample 25 µg/ml	35.63	31.83	29.30	27.75
Control + Test sample 50 µg/ml	39.51	36.98	36.14	31.20
Control + Test sample 100 µg/ml	58.81	55.12	54.70	52.52

Table.3

Hydroxyl radical scavenging activity of EVME and EVAE

Control + Test sample 10 µg/ml	20.74	15.20	14.39	9.75
Control + Test sample 25 µg/ml	35.63	31.83	29.30	27.75
Control + Test sample 50 µg/ml	39.51	36.98	36.14	31.20
Control + Test sample 100 µg/ml	58.81	55.12	54.70	52.52

*Each value represents mean±SEM (n=3)

Table.4

Total antioxidant capacity, flavonoid content, and phenolic content of EVME and EVAE

Name of extracts	Total antioxidant capacity (mg/g)	Total flavonoid content (mg/g; of quercetin equivalent)	Total phenolic content (mg/g)
EVME	1.74±0.39	1.98±0.12	110±0.32
EVAE	1.21±0.14	1.41±0.10	94±0.91