PHYTOCHEMICAL INVESTIGATION AND PHARMACOLOGICAL EVALUATION OF *ERYTHRAEARAMOSISSIMA*

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ABSTRACT

*Erythraearamosissima*is traditionally used for the treatment of various human diseases. In the present study was aimed at the *in-vitro* pharmacological and phytochemical analysis of the methanol extract of *Erythraearamosissima*. The sample extract of *Erythraearamosissima*showed potent antioxidant activities (98.1±5.2%) inhibition of DPPH, Hydrogen peroxide scavenging activity was (95.1±8.4%), total antioxidant activity was 92.1±5.4 and ABTS scavenging activity of the extract was 94.1±5.4 at 3000µg/ml.Qualitative analysis reveals the presence of saponins, tannins, anthraquinons, alkaloids, coumarins and flavonoids.I the cytotoxicity assay, at 3000µg/mlconcentrations maximum death of brine shrimp was reported. In the light of the present result it is inferred that methanolic extract of *Erythraearamosissima* can be used as a free radicle scavenger and as an anticancer agent.

Keywords:-*Erythraearamosissima*, scavenging activity DPPH, ABTS, phenolics, flavonoid compounds, cytotoxicity.

INTRODUCTION

Various free radicals are produced through the oxidative processes within the human body. The excess of free radicals and reactive oxygen species induce cellular damage and are involved in several human diseases such as diabetes mellitus, cancer and others inflammatory disorder. Recently several herbal formulations that have free radical scavenging potential are used in treating such chronic diseases (Wang et al., 2005). Medicinal plants are rich in various secondary metabolites, including but not limited to polyphenols and flavonoids, which are capable of eliminating free radicals (Manach et al., 2005). Polyphenols are present in the plant raw material having significant antioxidant properties. The antioxidant activity of polyphenols is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers (Prior et al., 2005). The anti-oxidative phytochemicals found in medicinal plants have received increasing attention for their potential role in prevention of human diseases (Vinary et al, 2004) and still there is a demand to find more potent antioxidants of plant species, as they are safe and also bioactive. Therefore recently considerable attention has been given to the identification of plants with antioxidant ability (Miller, 1996). With this work, we aimed to contribute the understanding and discovery of powerful antioxidants for safe use of this herb for treatment of ailment in which free radicals are implicated. Medicinal plants play fundamental role in the maintenance of the standard of human wellbeing and health improvement. Diverse parts of medicinal plants have been playing very basic role in the human health. From a longtime, they have paid their services for human health and served humans in every field of life like dyes and medicines (Tyler, 1994). Medicinal plants have some biologically vigorous compounds such as flavonoids, saponins, steroids, vitamin C, cardiac glycosides, carotenoids and phenolic compounds which raise the immune system of the body. According to Bruneton (1995), the anti-inflammatory and antioxidant properties are due to their phenolic and flavonoids compounds. In drug discovery, there are two major approaches i.e. unsystematic/random screening and ethnomedical knowledge which are generally used for the assortment of plants species for therapeutic purposes and the ethno medical policy because of conventionally therapeutic uses are of great importance (Pieters and Vlietnick, 2005). The present study is arranged to find out its pharmacological basis for different activities.

Plant collection: Fresh leaves of *Erythraearamosissima* was collected from District Bannu, Khyber Pakhtunkhwa, Pakistan. Plants were identified by a prominent taxonomist, Professor Abdurrahman, Chairman, Department of Botany, Government Post Graduate College Bannu and verified by Prof. Dr. Sultan Mehmmod, Dean of Sciences, University of Science and Technology, Bannu. Plants specimens were submitted in the herbarium of University of Science and Technology Bannu, for future correspondence under specimen vouchers GH- 101. The plants material were rinsed by the distilled water and shade dried at room temperature (25ºC) for three weeks, ground into fine powder mechanically of net size 1 mm around.

Extract preparation: Approximately 1 kg plant powder was socked in 1.5 liter of 70% methanol (CH_3OH)by casual trembling. After 96 hours, the extract was filtered by using Whatman filter paper No.1 and filtrate was

further contemplated viarotary vacuum evaporator at 38° C in order to get the methanolic crude extract of the plant. The methanolic crude extract was stored up at 4°C in the refrigerator for additional phytochemical studies and invitro examination.

Qualitative phytochemical screening: Phytochemical screening was performed to perceive the presence of bioactive compounds in plant extract through standard phytochemical methods.

Cytotoxic brine shrimp assay: Stock solution (5mg/ml) of methanol crude extract of *Erythraearamosissima* was prepared in methanol for this cytotoxic brine shrimp assay. From stock solution, various concentrations (375µg/ml, 750µg/ml, 1500µg/ml, and 3000µg/ml) of extract were prepared using the formula M1V1 = M2V2.2.8g of marketable marine salt (Sigma) was dissolved in 100 ml of distilled water with constant shaking for two hrs.Medium was put into two section rectangular tray enclosing huge number of small size holes for the passage of larvae. Eggs of *Artemiasalina* were sprinkled in dark compartment of tray and were located under the table lamp for incubation for 24 hrs. After 24 hrs, the larvae were emerged and migrated to the lightened side by way of holes and thus they were made together by pasture pipette.

In this attempt, drum vials were used in duplicate. 0.5 ml (500µl) of each sample solution (375µg/ml, 750µg/ml, 1500µg/ml, and 3000µg/ml) was obtained in vials and faded away the methanol solvent completely because of its venomous chemical character. The outstanding in the vials were dissolved in 2 ml of the sea salt solution. 10 brine shrimps were transferred to each vial by pasture pipette both in model treatedand in the control vials and raised the volumes up to 5 ml in each vial by using sea salt solution. In the control vials, only the sea salt solution was present. All the vials were packed forcefully and incubated at 28 C° for 24 hrs. After the incubation of 24 hrs, thenumber of survivors was calculated by 3x magnifying glass and the computation was made according to the Abbot's formula i.e.

% Death = (Sample-Control/Control) x 100.

Antioxidant Assay

DPPH free radicals scavenging assay: For this attempt of DPPH (1, 1-diphenyle -2- picrylehydrazyl) scavenging activity, the technique of Gymfi *et al*., (1999) with some modifications.

Phosphomolybdate assay (Total antioxidant capacity assay): The antioxidant activity of samples was evaluated by the phosphomolybdenum method according to the procedure of Umemura et al., (1995). An aliquot of 0.2 ml of sample solution was mixed with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The test tubes were capped with silver foil and incubated in a water bath at 95 ºC for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 765 nm against a blank. Ascorbic acid was used as standard. The antioxidant capacity was estimated using following formula:

Antioxidant effect (%) = [(control absorbance-sample absorbance) / (control absorbance)] ×100.

Hydrogen peroxide-scavenging activity: The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of Ruch*et al*. (1989). A solution of hydrogen peroxide (2 mM) was prepared in 50 mM phosphate buffer (pH 7.4). Aliquots (0.1 ml) extracts sample was transferred into the test tubes and their volumes were made up to 0.4 ml with 50 mM phosphate buffer (pH 7.4). After addition of 0.6 ml hydrogen peroxide solution, tubes were vortexed and absorbance of the hydrogen peroxide at 230 nm was determined after 10 min, against a blank. The abilities to scavenge the hydrogen peroxide were calculated using the following equation:

Hydrogen peroxide scavenging activity = (1- absorbance of sample / absorbance of control) × 100

ABTS radical cation scavenging activity: The ABTS (2, 2 azobis, 3-ethylbenzothiozoline-6-sulphonic acid) radical cation scavenging activity was performed by a slight modification of Re *et al*. (1999). Briefly, ABTS solution (7 mM) was reacted with potassium persulfate (2.45 mM) solution and kept for overnight in the dark to yield a dark colored solution containing ABTS radical cation. Prior to use in the assay, the ABTS radical cation was diluted with 50% methanol for an initial absorbance of about 0.700 (±0.02) at 745 nm, with temperature control set at 30 °C. Free radical scavenging activity was assessed by mixing 300 µl of test sample with 3.0 ml of ABTS working standard in a micro cuvette. The decrease in absorbance was measured exactly one minute after mixing the solution, then up to six minutes. The final absorbance was noted. The percentage inhibition was calculated according to the formula:

Scavenging effect (%) = [(control absorbance-sample absorbance) / (control absorbance)] × 100

The antioxidant capacity of test samples was expressed as EC_{50}, the concentration necessary for 50% reduction of ABTS.

Statistical analysis: MS Excel, Graph Pad Prism software were used for analysis.

RESULTS

Biochemical analysis: Medicinal plants have preventive potential due to the presence of secondary metabolites. Biochemical study of the plant extract attained with organic solvent is intense significant concern to diverse

diseases. In the present study, methanolic extract of *Erythraearamosissima* was characterized due to their traditional importance in different diseases. Phytochemical screenings of the methanolic extract indicates the presence of different ingredients which are presented in the Table 1. Saponins, tannins, terpenoids, alkaloids, steroids, flavonoids, and phenolic compounds are present while cardiac glycosides are absent in the methanolic extract of *Erythraearamosissima*.

Invitro Bioassays

Cytotoxic brine shrimp assay: Crucial displaying of the plant through cytotoxicity supplies positive information's about the anti-cancer activity of the plant's extract for the expectations use. Cytotoxic effect of the *Erythraearamosissima* was considered and noted along side's sea water growth of shrimps as shown in Tables 2. From the Table 2, it is clear that cytotoxic effects of methanolic extract of *Erythraearamosissima* is directly proportional to concentration i.e. at 3000µg/ml 0% survival and 100% death occurred.

Antioxidant Activity: Four different free radicals assays for antioxidant potential of *Erythrearamosissima* were performed i.e. against DPPH, total antioxidant, H_2O_2 and ABTS, the results of which were as under.

DPPH free radicals scavenging activity: 1,1- diphenyl 2- picryl – hydrazyl (DPPH) i.e. the free radicals have the ability to take electrons from the anti-oxidants, that is why it is widely used for the in vitro antioxidants foraged assays of the medicinal plants i.e. for its judgment purposes. Table3 shows that % scavenging activities of the *Erythrearamosissima* as compare to ascorbic acid which is utilized as standard. It is noted that the order of scavenging activity of *Erythrearamosissimaare* 375 µg/ml < 750 µg/ml < 1500 µg/ml < 3000 µg/ml.

Table 1. Qualitative phytochemical composition of *Erythraearamosissima.*

Samples	Saponins	Tannins	Anthraquinons	Alkaloids	Coumarins	Flavonoids	Cardiac glycosides	Phenolic compounds
ERME	+	+	+	+	+	+	-	+

"+" means presence while "-" means absence of the compounds.

Table 2. Survival and death of brine shrimps in the presence of various concentrations of *Erythrearamosissima*

Concentration	% Survival	% Death
375µg/ml	40.5±1.9	60.2±3.5
750µg/ml	30.6±2.0	70.3±1.7
1500µg/ml	20.3±1.1	80.7±3.2
3000µg/ml	0.0±0.0	100.0±0.0

Mean± SEM

Table 3. % DPPH free radicals scavenging activity of *Erythrearamosissima.*

Concentration	*Menthasylvestris*	ascorbic acid
375	45.0±2.3	73.0±3.6
750	55.5±3.2	81.5±5.2
1500	78.6±1.5	90.5±4.3
3000	88.0±3.5	98.1±5.2

Mean±SEM

ABTS radical scavenging activity of extract: Table 4 shows that the ABTS radical scavenging ability of samples i.e. *Erythrearamosissima*can be ranked as of *are* 375 µg/ml < 750 µg/ml < 1500 µg/ml < 3000 µg/ml. The results obtained clearly imply that all the tested samples inhibit or scavenge the radical in a concentration dependent manner. The values obtained from ERME and ascorbic acid is presented below.

Phosphomolybdate assay (Total antioxidant capacity) of extract: Table 5 depicts, the total antioxidant capacity of different fractions of methanol extract of samples i.e. *Erythrearamosissima*can be ranked as375 µg/ml < 750 µg/ml < 1500 µg/ml < 3000 µg/ml. However, the scavenging activity of all the extracts was found to be low when compared to ascorbic acid.

Table 4. % ABTS free radicals scavenging activity of *Erythrearamosissima.*

Concentration	*Erythrearamosissima*	ascorbic acid
375	34.5±3.7	53.0±3.2
750	55.3±6.4	61.5±3.4
1500	67.9±3.4	73.6±3.7
3000	81.0±7.4	94.1±5.4

Mean±SEM

Table 5. % total antioxidant activity of *Erythrearamosissima*

Concentration	*Menthasylvestris*	ascorbic acid
375	24.9±2.5	53.0±3.7
750	35.7±3.6	68.5±5.1
1500	58.3±5.3	77.5±4.7
3000	78.5±4.7	92.1±5.4

Mean±SEM

Hydrogen peroxide radical scavenging activity of extract: The scavenging effect of the extracts on hydrogen peroxide is shown in Table 6. As compared with values the hydrogen peroxide- scavenging activities of MSME were more effective than that of ERME.

Table 6. Hydrogen peroxide free radicals scavenging activity of *Erythrearamosissima*

Concentration	*Erythrearamosissima*	ascorbic acid
375	42.0±4.3	53.0±3.4
750	54.5±4.4	71.5±4.5
1500	75.6±6.5	90.5±4.4
3000	84.0±5.5	95.1±8.4

Mean±SEM

DISCUSSION

*Erythrearamosissima*in the every living systems for the fabrication of energy "oxidation" is one of the important and obligatory route, however in this oxidation procedure (oxygen utilization during normal metabolism) the RFR (reactive free radicals) are formed by various enzymatic systems. The ROS (reactive oxygen species) in minute amounts are beneficial for growth regulation and signal transduction, but ROS in large amounts produce oxidative stress which attacks and damage various molecules like DNA, Protein and Lipids (Halliwell and Gutteridge 1999).

The obtained data from the present methanolic extracts show significant scavenging potential. The anti-oxidant potential of the methanolic extracts of the medicinal plants are due to the phenolic and polyphenolic compounds present in them, which markedly reduce the free radicals that cause the oxidative stress (damage the molecules). Our results show some similarities with the investigation of Hagerman *et al*., (1998) and Falleh *et al*., (2008), who reported that free radicals are markedly scavenge by medicinal plants. The results obtained from our experiments are also supported by the findings of Duenas *et al*., (2006) and Kilani*et al*., (2008).

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