International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 5 Issue 5, July-August 2021 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

Qualitative Phytochemical Screening and *In-Vitro* Assessment of Antioxidant and Anti-Inflammatory Potential of Aqua Methanol and Aqua Acetone Extract of *Cirsium Arvense* and *Erigeron Bonariensis*

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ABSTRACT

In the present study, two plant species of the family Asteraceae were selected for an evaluation of their phytochemical screening, antioxidant and anti-inflammatory properties. Phytochemical alkaloids, phenols, protein, flavonoids, quinines, tannins, and terpenoids are present in the aqua methanol and aqua acetone extract (s) of *Cirsium arvense* (L) Cronquist and *Erigeron bonariensis*. The selected plant species exhibit anti-inflammatory properties in both solvents. The enzymatic antioxidant property of selected plant species was evaluated by superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT). *E. bonariensis* shows (20.05±0.02) superoxide dismutase activity which is moreover equal to *C. arvense* (19.47±0.31). POD and CAT activities of *C. arvense* (109.35±0.69 and 41.48±0.13) and *E. bonariensis* 105.91±1.53 and 39.63±0.035 respectively, the POD activity of *C. arvense* is slightly higher than *E. bonariensis* but CAT activity again higher in *E. bonariensis* same as SOD.

KEYWORDS: *Phytochemical Screening, Antioxidant, Anti-inflammatory, Medicinal Plants, Asteraceae*

How to cite this paper: Deepti Rawat | P. B. Rao "Qualitative Phytochemical Screening and *In-Vitro* Assessment of Antioxidant and Anti-Inflammatory Potential of Aqua Methanol and Aqua Acetone Extract of *Cirsium Arvense* and *Erigeron Bonariensis*" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-5 | Issue-5, August 2021, pp.724-728, URL: www.ijtsrd.com/papers/ijtsrd43934.pdf

IJTSRD43934

INTRODUCTION

Plants are a multi-cellular eukaryotic, photosynthetic organism, and being used for multiple purposes by humans since the primeval era. A large number of the world population depends on the plants for food, fiber, paper, wood, medicine, and rubber, etc. The plants that carried pharmacological propitious potential on the living system are categorized as Medicinal Plant. The availability of secondary metabolites such as flavonoids, terpenoids, and alkaloids exerts pharmacological properties in medicinal plants. Medicinal plants playing a very significant role in the health care of human beings. Pharmaceutical companies are predominantly concerned in these bioactive compounds, because of their remedial property and minor lethal effects [2] In the present scenario, one of the most common reasons for pernicious diseases is oxidative stress. Free radicals which are produced in the living system during different metabolic reactions, and environmental stresses (biotic and abiotic), in low concentration they act as signaling molecules but in high concentration, responsible for creating oxidative stress in the human body, antioxidants are those bioactive compounds that can be protected our body from reactive oxygen species (ROS) induced stress [12, 13]. *Cirsium arvense* belongs to the family Asteraceae (Compositae), commonly called creeping thistle, a perennial herb, and native to north-east regions of Europe and Asia continent [23]. It endowed with various ethnomedicinal potential, including a

diuretic, hemostatic and anti-inflammatory property in folk remedies [24, 30]. It also has other biological properties, such as antioxidants [22] and antimicrobial [21, 5]. *Erigeron bonariensis*, (Synonym *Conyza bonariensis*), an annual herbaceous weed, commonly called hairy fleabane, is another plant species selected for the present investigation. It is also belonging to the family Asteraceae.

There are only a few studies reported enzymatic antioxidant and anti-inflammatory potential of *C. arvense* and *E. bonariensis*. Therefore the aim of the present research was to evaluation of the anti-inflammatory potential of aqua acetone and aqua methanol extract (s) of *C. arvense* and *E. bonariensis* and possible enzymatic antioxidant property of their fresh leaves. The main criteria for the selection of these plant species were previously reported non-enzymatic antioxidant property as well as their ethnomedicinal importance.

MATERIALS AND METHODS

Sample collection and preparation of extract

Plant material *C. arvense* and *E. bonariensis* based on their ethnomedicinal importance collected from the campus and nearby area of G.B Pant University, Pantnagar in March-April 2019. We removed leaves from aerial parts, washed them under tap water multiple times. Some of the fresh leaves (approximately 10gm) were kept for the evaluation of enzymatic antioxidants, and the remaining sample was air-dried for 8-10 days at room temperature, and then ground to a fine powder in using an electrical grinder. For the extraction, the maceration method was used with the required modification [6].

The sample was prepared by soaking 10 gm of plant material in 250 ml flask with 100 ml of 80 % aqua acetone and aqua methanol using a rotator shaker at 120 rpm for 10 days, after completion of the time of extraction, the extracted sample was filtered and kept for evaporation of the solvent. Finally obtained a greasy product was kept at -4°C for further evaluation of anti-inflammatory potential.

Phytochemical screening

Phytochemistry screening of aqua methanol and aqua acetone extract of *C. arvense* and *E. bonariensis* was performed by applying methodologies of [11, 28, and 14]. Major bioactive compounds analyzed were carbohydrates, alkaloids, phenols, protein, flavonoids, quinines, tannins, saponins, steroids, cardiac glycosides, and terpenoids. These bioactive compounds display very important roles in the growth and development of the plant.

Protein estimation

The protein content of the fresh plant sample was estimated by using the method proposed by [18] with suitable modifications.

Enzymatic antioxidants

Enzymatic antioxidant activity of selected plant species was evaluated according to the method of [10] for superoxide dismutase activity (SOD), [15, 33] for Peroxidase activity (POD), and [15] for with required medications. These following equations were used for the calculation of SOD and POD activities. Z=(X-A) X×100

Where Z= photo-inhibition % of the sample; X= absorbance of control; A= absorbance of enzyme sample.

U/ml enzyme= (At/min-Ao/min)12×0.1×3×df

Where, At =Absorbance of test sample; Ao =Absorbance of control; 3 = Volume (ml) of reaction mixture; df = dilution factor; 12 = Extinction coefficient of 1 mg/ml of pupurogallin; and 0.1 = Volume (ml) of the test sample.

Evaluation of in vitro anti-inflammatory activity

An *in-vitro* anti-inflammatory potential of aqua methanol and aqua acetone extracts of selected plant species was evaluated by using a methodology of [26] with required modifications.

Data presentation and analysis

Statistical analysis was performed using Microsoft Office Excel 2007. The significant differences among different groups of data were evaluated with ANOVA by using SPSS 16.0 and Duncan's multiple range tests; the level of significance was $P < 0.05$. All outcomes of experiments were expressed as mean and standard error (mean ± SE), calculated from triplicate.

RESULTS AND DISCUSSION

Extraction yield and phytochemical screening

The extraction yield of *C. arvense* and *E. bonariensis* in aqua methanol were 13.12±0.06 % (w/w) and 22.54±0.53% (w/w), while in aqua acetone 9.55±0.20 % (w/w) and 14.07±0.51 % (w/w) respectively. In aqua methanol extraction yield is higher as compared to aqua acetone, it might be due solubility of more phytochemicals in methanol. Phytochemicals or available concentration of bioactive compounds exerts pharmacological property in plants, triterpenoids, reducing sugars, gums, flavonoids, tannins, and xanthoprotein provide analgesic, antibiotic and anticancerous potential [1, 3, 32]. Phytochemical groups of alkaloids, flavonoids, tannins, and terpenoids reported having antimicrobial property [20, 31]. Saponins display hypocholesterolemic and anti-diabetic properties [3].

In the current study aqua methanol and aqua acetone extracts of both plant species were analyzed, the major phytochemicals alkaloids, carbohydrates, flavonoids, phenols**,** proteins, quinines, tannins, and terpenoids were present in both extracts of *C. arvense* and *E. bonariensis*. Whereas cardiac glycosides were absent in both extracts of *E. bonariensis* and steroids in *C. arvense*. Among the all eleven selected phytochemical saponins were the only the absent in both extracts of selected plant species. Results of extraction yield and phytochemical screening are shown in **(Table 1).** The availability and concentration of a phytochemical in any plant species depend on the genetic constitution, climatic conditions, geographical regions, solvent, and method of extraction.

Anti-inflammatory potential or inhibition of protein denaturation

The results of the current study indicate both selected plant species *C. arvense* and *E. bonariensis* exhibit anti-inflammatory properties in both aqua methanol and aqua acetone extracts. The anti-inflammatory property was determined by using the denaturation of egg albumin with different concentrations (50, 100, 150, 200, 250, and 300µg/ml). The results of protein denaturation are shown in **(Fig 1 and Fig 2).** In aqua methanol, *C. arvense* display significantly higher antioxidant property than *E. bonariensis*, whereas in aqua acetone both the plant species exhibit moreover similar activity. But if we compare inhibition activity between the solvent, aqua methanol shows higher anti-inflammatory properties as compared to aqua acetone, it might be due to more effective bioactive compounds dissolve in aqua methanol, responsible for anti-inflammatory property. Considerable differences were observed in the inhibition of protein denaturation activity at six different concentrations in both the plant species. The petroleum ether and ethanolic extract of *Erigeron canadensis* showed a significant anti-inflammatory effect on a rat with a carrageenan and formalin edema [4]. The main bioactive compounds responsible for the anti-inflammatory property are alpha-curcumin, beta-santalene, beta-himachalene, gamma-cadinene, cuparene, and three others [17].

Protein content

Protein content in fresh leaves of *C. arvense* and *E. bonariensis* was evaluated to determine the enzymatic antioxidant property. Results show protein content in *E. bonariensis* (40.36±0.58) is higher than *C. arvense* (33.29±0.74) **(Fig. 3).**

Enzymatic antioxidant

Antioxidants protect the living organism from various stresses, they provide protection either by suppress or delay the action of stress. The plant produces several enzymes to protect their body from exogenous or endogenous stresses. Superoxidase dismutase, glutathione peroxidase, catalase, and glutathione S-transferases (GSTs) are the major enzymes produces by plants. Most of the isoenzymes of glutathione S-transferases (GSTs) are found in the cytosol of a cell, play a major role in detoxification [16], and show an active role as an inhibitor for mitogen-activated protein kinase during the development of drug resistance [25, 29, 9]. Glutathione peroxidase is another important enzyme that protects the cell by participating in oxidative stress. The way of action of peroxidase enzyme is the reduction of peroxides, such as hydrogen peroxide [16]. Superoxide dismutase (SOD) catalyzes reactive oxygen species into an oxygen molecule and hydrogen peroxide [7]. Catalase enzyme catalyzes the reaction initiated by superoxide [19].

Several studies demonstrated that many members of the family Asteraceae exhibits pharmaceutical properties because of the presence of polyphenols, flavonoids, and diterpenoids bioactive compounds [8, 27]**.** In the present investigation both plant species exhibit considerable enzymatic antioxidant activity, a very less significant difference was observed between the both selected plant species. The fresh leaves of *E. bonariensis* show (20.05±0.02) superoxide dismutase activity which is moreover equal to *C. arvense* (19.47±0.31). POD and CAT activities of *C. arvense* (109.35±0.69 and 41.48±0.13) and *E. bonariensis* 105.91±1.53 and 39.63±0.035 respectively, the POD activity of *C. arvense* is slightly higher than *E. bonariensis* but CAT activity again higher in *E. bonariensis* same as SOD. The results of enzymatic antioxidants are shown in **(Fig. 4, 5, and 6).**

Conclusion

The current study reports screening of phytochemicals, enzymatic antioxidant and anti-inflammatory potential of *C. arvense* and *E. bonariensis*, agricultural weeds from the kumaun region of Uttarakhand, India. Aqua methanol and aqua acetone extract showed alkaloids, carbohydrates, flavonoids, phenols**,** proteins, quinines, tannins, and terpenoids, *in-vitro* anti-inflammatory activity. Fresh leaves of both plant species showed a significant amount of SOD, POD, and CAT activities. Phytochemical screening, antioxidant, and anti-inflammatory results itself show advantages for the present and future pharmaceutical companies.

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