A COMPARISON OF BIOACTIVE CONSTITUENTS AND IN VITRO ANTIOXIDANT POTENTIAL OF ASTHMA WEED (*EUPHORBIA HIRTA*), WITH THOSE OF OTHER ANTI-ASTHMATIC PLANTS GROWING IN TABUK REGION, SAUDI ARABIA

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Abstract. *Euphorbia hirta* (asthma weed) has been documented as an effective herb for the treatment of asthma and associated symptoms. This study was aimed at comparing the bioactive constituents and *in vitro* antiradical activity of asthma weed with other commonly growing anti-asthmatic plants such as *Pergularia tomentosa*, *Dipterygium glaucum* and *Acalypha indica* in Tabuk region of Saudi Arabia. The results showed that the total phenol and flavonoid content of methanolic leaf extract of *E. hirta* was highest compared to the other anti-asthmatic plants. *E. hirta* was also recorded to have the maximum total antioxidant capacity, 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) antiradical activity and nitric oxide (NOX) scavenging activity. However, hydrogen peroxide scavenging activities and metal chelating activities were as the highest for the methanolic extracts of *D. glaucum* and *P. tomentosa*, respectively compared to the other anti-asthmatic plants. It may be concluded that the abundance of phenol and flavonoids along with maximum antioxidant capacity, DPPH antiradical activity and NOX scavenging activity of *E. hirta* makes it worthy of using as an effective anti-asthmatic plant.

Keywords: *flavonoids, oxidative stress, antioxidant capacity, antiradical scavenging activity, phenols*

Introduction

Asthma is a chronic lung disease, inducing narrowing of airways and changes in the levels of eosinophils, mast cells, lymphocytes, cytokines and other inflammatory cell products (Rivera et al., 2011). Asthma is a common chronic disease that is estimated to affect as many as 339 million people worldwide (WHO, 2018). However, Bousquet et al. (2005) predicted that 100 million more will be affected by asthma by 2025. It causes a high global burden of death and disability, with around 1000 people dying each day from asthma, and is in the top 20 causes of years of life lived with disability (WHO, 2018). Nearly 2 million people are affected by asthma in the Kingdom of Saudi Arabia. There are no complete cures of asthma and currently available modern therapies have been associated with many side effects. So, to find an effective low risk, a non-drug strategy that may furnish a valuable adjunctive or alternative therapy in asthma management is both alluring and timely.

Of late, medicinal plants have attracted a huge interest due to the inherent antioxidant potential of the phytochemicals that reduce the free-radical induced oxidative damage. It is estimated that 80% of the world population rely on drugs obtained from plants, for their primary health care needs (WHO, 2019). It has been established that climate change may affect the chemical composition and, ultimately the survival of some medicinal plants. Climate change has a remarkable effect on secondary metabolites and other compounds that plants produce, which are usually the basis for their medicinal

activity (Khan et al., 2016; Yang et al., 2018). Phytochemical screening by several workers has unveiled the presence of various bioactive constituents such as phenols, flavonoids, alkaloids, terpenoids and saponins (Altemimi et al., 2017; Verma et al., 2018; Sai et al., 2019; Batool et al., 2019). Medicinal plants that may be employed for the treatment of asthma should have anti-allergic, anti-inflammatory, antihistaminic and immunomodulatory properties. It has been observed that the supplements with antioxidant properties are potentially effective in decreasing the degree of bronchoconstriction as they suppress the pro-inflammatory episodes by counteracting reactive oxygen species and reactive nitrogen species (Kurutas, 2016; Bagatini et al., 2018).

Tabuk, the northern province of Saudi Arabia (Fig. 1) is a region largely characterized by asymmetrical topography ranging from plains to low and high mountains that create a highly distinct environmental variable of extreme cold to extreme hot (Khan et al., 2016). Diversified topography coupled with varied environmental conditions supports the growth of several medicinally important plants in this region. Of these, *Euphorbia hirta* (Euphorbiaceae), *Pergularia tomentosa* (Asclepiadaceae), *Dipterygium glaucum* (Capparidaceae) and *Acalypha indica* (Euphorbiaceae) have long been used in traditional medicine to treat asthma (Kumar et al., 2010; Youssouf et al., 2007; Zahidin et al., 2017; Alamgeer et al., 2018; Rayyan et al., 2018). *Euphorbia hirta* is known as asthma weed, spurge or milkweed and used to treat respiratory complications. Although, various studies have been performed on the phytochemical constituents of these plants, but meagre research was carried out on the comparative study of antioxidant potential of these plants. Therefore, the objective of the current study was to compare the antioxidant potential and secondary metabolites of asthma weed with other anti-asthmatic plants found in Tabuk region.

Figure 1. Map showing sampling site (Tabuk, Saudi Arabia). (Source: Google images)

Materials and methods

Plant collection

The plants were collected from Tabuk, the Northern Province of Saudi Arabia (latitude 28°22'59" N; 36°34'59" E, altitude 773 m). The region is characterized by its

hyper-arid climate, high evaporation rate and low precipitations during December to February. The region is bounded by Red sea on the west to the Hufa depressions in the east. Four plant species (*E. hirta*, *P. tomentosa*, *D. glaucum* and *A. indica*) were collected at flowering stage. At collection site five 10 m × 10 m plots, at the distance of 20 m each, were established randomly during the study. Four plants of each species from each plot were collected randomly. Finally, five healthy plants of each species were selected and were used for assessing various characteristics. The plant materials were authenticated by Dr. M. Nasir Khan. Collected plants were dried in shade under dark. Air-dried leaf samples were ground to a fine powder (80 mesh) using an electric blender and stored in a clean labeled air-tight container as replicates for assessing various characteristics.

Plant extracts

Powdered leaf sample (100 g) of each plant was extracted with methanol for 24 h using Soxhlet apparatus (Borosil, BRL_3840020). The extracts were separated from the solids by filtration with Whatman No. 1 filter paper. The remaining solids were extracted twice with the same methanol and extracts combined. The extracts were concentrated under reduced pressure at 45 °C in a rotary evaporator (EYELA, Tokyo, Japan) and kept in a refrigerator at 4 °C until analyzed.

Estimation of total phenol

The content of total phenols was estimated by the Folin-Ciocalteu method with little modification of Cevallos-Casals and Cisneros-Zevallos et al. (2005). From each sample, 0.5 ml of methanolic extract was added to 2.5 ml of Folin-Ciocalteu reagent and 2 ml of sodium carbonate 1 M. The tubes were incubated at 45 °C for 30 min. The absorbance of total phenolics was measured at 765 nm using Hewlett Packard, UV/visible light. Total phenolics content was expressed as mg gallic acid equivalents per g dry weight.

Estimation of total flavonoids

Total flavonoid content in the methanolic extracts was measured spectrophotometrically (CE 2021, Cecil, Cambridge, England), following the method of Marinova et al. (2005). 4 ml of water was added to 1 ml (500 mg/ml) extract or standard catechin solution and 0.3 ml of 5% NaNO₂. After keeping it for 5 min, 0.3 ml 10% AlCl₃ was added. To this mixture, after 6 min 2 ml 1 M NaOH was added and the total volume was made up to 10 ml with water. The solution was thoroughly mixed, and the absorbance was recorded against a prepared reagent blank at 510 nm. Total flavonoid content of was expressed as catechin equivalents in mg per g dry weight.

Assay of total antioxidant capacity

Total antioxidant capacity was determined by the method described by Prieto et al. (1999). 0.1 ml of methanolic plant extract was added to 1 ml of reagent solution (0.6 mol/l sulfuric acid, 28 mmol/l sodium phosphate and 4 mmol/l ammonium molybdate). The tubes were heated at 95 °C for 90 min. The absorbance of each solution was recorded at 695 nm against a blank. The antioxidant capacity was expressed as mg gallic acid equivalent per gram dry weight (mg GAE/g DW).

Anti-radical capacity

Determination of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging

The free radical scavenging capacity of methanolic extracts of different plants was recorded using DPPH method as described by Thaipong et al. (2006). 5 ml of 0.004% freshly prepared methanolic solution of DPPH (2, 2-diphenyl-1-picrylhydrazyl) were added to 50 μ l of different concentrations of sample. After 30 min in the dark at room temperature, the absorbance was recorded spectrophotometrically against a blank at 517 nm. DPPH free radical scavenging activity was expressed as the percentage inhibition.

Nitric oxide (NOX) scavenging activity

To estimate NOX radical inhibition activity, method described by Marcocci et al. (1994) was followed with little modifications. Briefly, sodium nitropruside (5 mM, pH 7.4) in phosphate buffer saline was mixed with 3 ml of different concentrations of methanolic plant extracts and incubated at 25 °C for 150 min. From this incubated solution, 0.5 ml was taken and mixed with 0.5ml Griess reagent [(1.0 ml sulfanilic acid reagent (0.33% in 20% glacial acetic acid at room temperature for 5 min with 1 ml of naphthylethylenediamine dichloride (0.1% w/v)]. After 30 min of incubation, absorbance was recorded at 540 nm. A standard solution of ascorbic acid was treated in the same way with the Griess reagent as a positive control. NOX scavenging activity was expressed as the percentage inhibition.

Hydrogen peroxide (H₂O₂) scavenging activity

The ability of the extracts to scavenge H₂O₂ was estimated based on the method of Ruch et al. (1989). A solution of H₂O₂ (40 mM) was prepared in phosphate buffer (pH 7.4). The concentration of H₂O₂ was determined by absorption at 230 nm using a spectrophotometer. Plant extracts in methanol were added to a H₂O₂ solution (0.6 ml, 40 mM). The absorbance of H₂O₂ at 230 nm was noted after 10 min against a blank solution containing phosphate buffer without H₂O₂. Ascorbic acid was used as positive control. H₂O₂ scavenging activity was expressed as the percentage inhibition.

Metal chelating activity

The formation of ferrous ion-ferrozine complex was recorded to estimate the ferrous level according to Decker and Welch (1990). The reaction mixture consisted of different concentration of plant extracts and 0.1 ml of 2 mM ferrous chloride. 0.2 ml of 5 mM ferrozine was added to this mixture to initiate the reaction and left to stand at room temperature for 10 min. The absorbance of the solution was measured at 562 nm. Ascorbic acid was used as positive control. The chelating activity was expressed as percentage of inhibition.

Statistical analyses

The data were analyzed statistically using analysis of variance (ANOVA), data were presented as average of five determinations with LSD at 5% $\pm$ SE. Differences between the means were compared by Duncan's multiple-range test at the level of $p < 0.05$ using SPSS Ver. 11 statistical software (SPSS Inc., Chicago, IL, USA). IC₅₀ values were calculated by employing linear regression analysis.

Results and discussion

Asthma is an inflammatory disease of the lungs and the present endeavour is to control the inflammation. As in *Table 1*, methanolic leaf extracts of *E. hirta*, *P. tomentosa*, *D. glaucum* and *A. indica* showed significant amount of phenolic compounds. The total phenol content of the investigated plants differed significantly. *E. hirta*, the asthma weed has 13.9%, 21.1% and 7.3% more phenol than the *P. tomentosa*, *D. glaucum* and *A. indica*, respectively. We have recorded a higher amount of total phenol in these anti-asthmatic plants than that of other anti-asthmatic plants such as *Matricaria recutita* (Al-Dabbagh et al., 2019) but lower than the *Helichrysum* species (Albayrak et al., 2010).

Table 1. Total phenols, flavonoids and antioxidant capacity of methanolic extracts of leaves of anti-asthmatic plants *Euphorbia hirta*, *Pergularia tomentosa*, *Dipterygium glaucum* and *Acalypha indica*

Anti-asthmatic plants	Total phenols (mg GAE/g DW)	Total flavonoids (mg CE/g DW)	Antioxidant capacity (mg GAE/g DW)
<i>Euphorbia hirta</i>	87.7 ± 4.2	74.3 ± 3.1	67.4 ± 3.2
<i>Pergularia tomentosa</i>	75.5 ± 3.1	51.7 ± 2.1	61.2 ± 2.9
<i>Dipterygium glaucum</i>	69.2 ± 2.9	45.7 ± 2.0	54.9 ± 2.3
<i>Acalypha indica</i>	81.3 ± 3.7	58.7 ± 2.7	63.7 ± 3.5
LSD at 5%	3.09	2.41	1.86

Average of five determinations is presented with LSD at 5% ± SE

Flavonoids, low-molecular weight polyphenolic plant secondary metabolites are known to play a role in prevention and management of asthma. Recent *in vitro* and *in vivo* studies on anti-allergic and anti-asthmatic properties of flavonoids have strongly indicated that a suitable intake of flavonoids may complement the dietary treatment and/or preventive strategy for asthma or other allergic diseases in humans (Tanaka, 2014; Tanaka et al., 2019). Perusal of the data shows a significant difference among the species in the level of flavonoids (*Table 1*). Higher level of flavonoids were noted in the asthma weed *E. hirta* (74.3 ± 3.1 mg CE/g DW) which was 30.4%, 38.5% and 21% higher than *P. tomentosa* (51.7 ± 2.1 mg CE/g DW), *D. glaucum* (45.7 ± 1.98 mg CE/g DW) and *A. indica* (58.7 ± 2.7 mg CE/g DW), respectively. Garcia-Larsen et al. (2018) have found a direct association between flavonoid intake and asthma prevalence and severity.

We carried out the phosphomolybdenum assay to determine the antioxidant capacity of methanolic leaf extracts of *E. hirta*, *P. tomentosa*, *D. glaucum* and *A. indica*. This method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of green Mo (V) complexes with a maximal absorption at 695 nm (Prieto et al., 1999). Analysis of variance showed that there was a significant difference among the studies plant species for antioxidant capacity. The methanolic extract of *E. hirta* had the maximum antioxidant activity with a value of 67.4 mg GAE/g DW. The minimum antioxidant capacity was recorded in the methanolic extract of *D. glaucum* with a value of 54.9 mg GAE/g DW (*Table 1*). Anti-asthmatic plants such as *Eriobotrya japonica* and *Tussilago farfara* showed a much higher antioxidant capacity than the plants which we studied (Song et al., 2010; Norani et al., 2019). Phytochemical

attributes such as flavonoids, carotenoids and cinnamic acid derivatives have been ascribed to contribute to the total antioxidant capacity (Tyśkiewicz et al., 2019). As, we know that oxidative stress is strongly linked with most of the features of asthma. The phytochemical preparations that are being able to scavenge these free radicals may help to relieve the symptoms of asthma. The free radical scavenging activities of methanolic leaf extracts of *E. hirta*, *P. tomentosa*, *D. glaucum* and *A. indica* were assessed by DPPH method. DPPH, stable free radicals that accept an electron or hydrogen radical and get converted in to yellow-coloured diphenylpicrylhydrazine, a diamagnetic molecule. The reduction capacity of DPPH is estimated by decrease in the absorbance at 517 nm induced by antioxidants. As shown in Figure 2, the DPPH radical scavenging activities of methanolic leaf extracts of different plants shown to occur in a dose-dependent manner. The concentration required to inhibit 50% radical-scavenging activity (IC_{50}) was established from the results of a series of concentrations evaluated. A lower IC_{50} value corresponds to a larger scavenging activity. The rank order of potency showed that the methanolic leaf extracts of *E. hirta* was 2.7-, 2.5- and 1.9 -fold more powerful than the

Figure 2. DPPH radical scavenging activities of the methanolic extracts of anti-asthmatic plants *Euphorbia hirta*, *Pergularia tomentosa*, *Dipterygium glaucum* and *Acalypha indica*. Average of five determinations is presented with LSD at 5% $\pm$ SE

P. tomentosa, *D. glaucum* and *A. indica*, respectively. The scavenging effect of methanolic extracts on the DPPH radical expressed as IC_{50} values was in the following order: *E. hirta* > *A. indica* > *D. glaucum* > *P. tomentosa* (Fig. 3). The substantial antioxidant activity of *E. hirta* could be due to the presence of sesquiterpene lactones and quercetin in flavonoids in the methanolic extracts (Williams et al., 2004; Gupta et al., 2017).

H_2O_2 is very lethal as being able to cross the biological membrane and serve as a precursor for potentially toxic hydroxyl radical (Das and Roychoudhury, 2014). The hydroxyl radical is an extremely reactive free radical capable of damaging almost every molecule found in living cells (Das and Roychoudhury, 2014). As in Figure 3, the methanolic leaf extracts of all the plants investigated, have shown a concentration

dependent H_2O_2 scavenging activity. Statistical analysis of the data shows a significant difference ($p < 0.05$) among the plants in relation to H_2O_2 scavenging activity. Lowest H_2O_2 scavenging activity was recorded for *E. hirta* which was 1.6-, 1.9- and 1.1-fold lower than *P. tomentosa*, *D. glaucum* and *A. indica*, respectively (Fig. 3). The H_2O_2 scavenging activity of methanolic extracts expressed as IC_{50} values was in the following order: *D. glaucum* > *P. tomentosa* > *A. indica* > *E. hirta* (Fig. 3). According to Mobin et al. (2014), H_2O_2 scavenging activity of these methanolic extracts could be assigned to the presence of active constituents that may donate electrons to H_2O_2 and thereby neutralizing it to water.

Figure 3. H_2O_2 scavenging activities of the methanolic extracts of anti-asthmatic plants *Euphorbia hirta*, *Pergularia tomentosa*, *Dipterygium glaucum* and *Acalypha indica*. Average of five determinations is presented with LSD at 5% $\pm$ SE

According to Terao (2009), NOX regulates many pathological conditions, especially acute inflammatory condition. Nitrite and peroxynitrite anions are generated when oxygen reacts with the excess NOX that may acts as free radicals (Radi, 2018). In this study, methanolic leaf extracts of all the investigated plants compete with oxygen to react with NOX and thus inhibits the generation of the anions in a dose dependent manner. As in Figure 4 the highest NOX scavenging activity among the analyzed plant extracts were displayed by *E. hirta* ($\text{IC}_{50} = 135 \mu\text{g/ml}$). We noted that the NOX scavenging activity of *E. hirta* was 1.2-, 1.4- and 1.1-fold more than *P. tomentosa*, *D. glaucum* and *A. indica*, respectively (Fig. 4). The bioactive constituents of *E. hirta* include flavonoids (quercetin and myrcetin), sterols, tannins, and triterpenoids (Attah et al., 2013; Onyeka et al., 2018; Salehi et al., 2019). The abundance of these bioactive substances makes them powerful scavengers.

As in Figure 5, the metal chelating ability of the methanolic leaf extracts of *E. hirta*, *P. tomentosa*, *D. glaucum* and *A. indica* were quantified by the formation of ferrous ion-ferrozine complex of red colour which absorbs at 562 nm (Yamaguchi et al., 2000). The ability of a chelating agent to form σ bond with a metal, may act as effective scavenger as they lower the redox potential and stabilize the oxidised metal ion (Duh et al., 1999). In the present study the plants show a significant difference response in metal chelating

activity. Highest and lowest metal chelating activity was noted in *P. tomentosa* and *D. glaucum*, respectively. Based on IC_{50} value, the rank order of potency for metal chelating activity of methanolic leaf extracts of all the samples were; *P. tomentosa* > *E. hirta* > *A. indica* > *D. glaucum* (Fig. 6). Fe^{2+} , a transition metal can transfer a single electron and can trigger a series of radical reactions even with non-transition metals. Fe^{2+} - chelating activity depends on flavonoid structures, however, the position of the hydroxyl ion in the molecule determines the proton donating and radical scavenging activity as suggested by Mira et al. (2002), Hou et al. (2003) and de Castilho et al. (2018).

Figure 4. Nitric oxide (NOX) scavenging activities of the methanolic extracts of anti-asthmatic plants *Euphorbia hirta*, *Pergularia tomentosa*, *Dipterygium glaucum* and *Acalypha indica*. Average of five determinations is presented with LSD at 5% $\pm$ SE

Figure 5. Metal chelating activities of the methanolic extracts of anti-asthmatic plants *Euphorbia hirta*, *Pergularia tomentosa*, *Dipterygium glaucum* and *Acalypha indica*. Average of five determinations is presented with LSD at 5% $\pm$ SE

Figure 6. IC₅₀ value (µg/ml) of DPPH radical scavenging, hydrogen peroxide (H₂O₂) scavenging, nitric oxide (NOX) scavenging and metal chelating activities of the methanolic extracts of anti-asthmatic plants *Euphorbia hirta*, *Pergularia tomentosa*, *Dipterygium glaucum* and *Acalypha indica*. Average of five determinations is presented with LSD at 5% ± SE

Conclusions

The findings of the present study have provided the biochemical evidences for the ethno-pharmacological applications of these anti-asthmatic plants. Based on statistical analysis of the data, it can be postulated that affluence of phenol and flavonoids along with maximum antioxidant capacity, DPPH antiradical activity and NOX scavenging activity in *E. hirta* makes it worthy of using as an effective anti-asthmatic plant. However, future work should be directed towards the isolation, identification and mode of action of bioactive constituents responsible for strong anti-asthmatic action of these plants.

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