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Research

Antioxidant Properties of Leaves Extracts of Acanthaceae Species

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ABSTRACT

The objective of this study is to determine the antioxidant properties of leaves extracts of 24 Acanthaceae species: *Asystasia guttata, Ballochia amoena, Barleria aculeata, B. orbicularis, B. parviflora, B. prionitis, B. ventricosa, Blepharis cayaniense, B. maderaspatensis, Crossandra, johanninae, Dicliptera effusa, D. paniculata, D. verticillata, Ecbolium gymnostachyum, Hypoestes pubescens, H. triflora, Justicia caerulea, J. heterocarpa, J. odora, Megalochalmyas violacea, Phaulopsis imbricata, Trichocalyx orbicular*, *Ruellia grandiflora* and *R. paulayana.* The radical scavenging activities of 2,2 diphenyl-1-picrylhydrazyl, ferric-reducing antioxidant power, and total phenolic compounds of species were determined using the Folin-Ciocalteau reagent. *Blepharis cayaniense* has the highest phenolic compounds (995.5 mg of gallic acid per g of sample) while *Hypoestes pubescens, and Ruellia paulayana* (10 mg/mL gallic acid equivalent) have the lowest concentration of phenolic acid but less radical scavenging activities (DPPH) and reducing ferric power. Only *Trichocalyx orbiculatus* has the highest concentration in phenolic content, radical-scavenging activities, and reducing power. Interestingly, the antioxidant activities of different Acanthaceae species exhibit unique medicinal properties.

Key words: Acanthaceae, antioxidant properties, phenolic content, radical-scavenging effect, reducing power

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INTRODUCTION

Acanthaceae is a large family of dicotyledonous flowering plants including about 4300 species and 346 genera all over the world. Most are tropical shrubs, herbs, and twining vines while some are epiphytes (Bhatt *et al*., 2010). It is widely distributed throughout the tropical regions of Africa, India, the Arabian Peninsula, and other parts of Asia (Athar *et al*., 2009). Many of the members of the Acanthaceae family are used traditionally as medication for wounds, asthma, anti-diarrhea, edema, pneumonia, treating skin diseases, cough, and eye infections (Madhu *et al*., 2010, Bremner *et al*., 2009; Bader *et al*., 2015). Antioxidants play a major role in the living system and prevent oxidative damage therefore, the search for naturally occurring antioxidants has greatly increased, focusing mainly on plants used in traditional medicine because the antioxidants play a key role in health, neutralizing reactive oxygen species (ROS) involved in human aging and diseases (Finkel & Holbrook, 2000; Siriwatanametanon *et al*., 2010).

ROS are continuously produced during regular physiological processes and they may cause cellular injuries, leading to the accumulation of lipid peroxides in biological membranes, damaging crucial biomolecules such as nucleic acids, lipids, proteins, polyunsaturated fatty acids, and carbohydrates. Oxidative stress leads to the pathogenesis of various lung disorders like asthma, chronic obstructive, pulmonary disorders, acute lung injury, and lung cancer. Most of the antioxidants present in a vascular plant such as vitamins C and E, carotenoids, flavonoids, and tannins. Hence, research should focus on improving the natural antioxidants from natural resources (Farombi & Fakoya, 2005; Hee *et al*., 2010)

According to Huang *et al*. (2013), plant polyphenols are one of the most numerous and widely distributed groups of natural antioxidants. It acts as reducing agents and antioxidants in vitro via several mechanisms including the scavenging of free radicals. Chemically it contained important secondary metabolites such as glycosides, flavonoids, alkaloids, triterpenoids, fatty acid methyl esters, and fatty acids. which give features as antioxidant, antimicrobial, anti-inflammatory, antitumor, and anticancer activity (Babu *et al*., 2002; Mani *et al*., 2008; Yahaufai *et al*., 2010; Thirunavukkarasu *et al*., 2011; Khlifi *et al*., 2013; Govindasamy & Arulpriya, 2013). These compounds play an important role in many biological reactions and work against many lethal diseases. Some studies such as (Yemane *et al*., 2017) referred to *Hypoestes forskaolii* belonging to the Acanthaceae family and has been used traditionally for the management of diabetes mellitus without scientific evidence of its safety or efficacy. However, ethnobotanical studies are often significant in revealing locally important plant species and providing important drugs of the modern day (Beyi, 2018). This medical importance of the Acanthaceae family attracted researchers to explore the various aspects of this family.

Ibrahim *et al*. (2017) presented an updated review of medicinal plants belonging to the family Acanthaceae. Abubacker and Kamala (2017) assessed *Lepidagathis sp* for its ethnomedicinal applications, phytochemical components, and pharmacological properties. A similar report on the phytochemical analysis and antibacterial activity of *Barleria pratensis* and *Barleria acuminata* was presented by (Renjini *et al*., 2017; Bency *et al*., 2018). In addition, Jara *et al*. (2017) evaluated the antioxidant activity of *Acanthus mollis,* and the result showed the highest concentration of phenols, anthraquinones, and flavonoids. Various species of *Barleria* reported as folk medicine, *Barleria longiflora* displayed nephroprotective activity (Manjula & Saravana, 2018a), *Barleria noctiflora* presented as anti-inflammatory (Manjula & Saravana, 2018b), and antidiabetic potential (Manjula & Saravana, 2018c). Neena *et al*. (2019) tested also antimicrobial activity of 4 traditional medicinal plants of Acanthaceae, another study made by Manjula and Anjalai (2019) showed the presence of glycosides, alkaloids, carbohydrates, tannin, phenolics, flavonoids, proteins, and amino acid in aerial parts of *Barleria buxifolia* L. (Acanthaceae).

Recently, Phumthum and Sadgrove (2020) described the traditional medicinal applications and evaluated phytochemicals and pharmacological activities of *Strobilanthus kuntianus* (Acanthaceae). Wakuma *et al*. (2021) showed that the methanolic leaf extract and solvent fractions of *H. forskaolii* have antidiabetic, and antioxidant activity that provides scientific support for the local use of the plant in the treatment of diabetes. Dhanalakshmi and Thangadurai (2021) and Ponnusamy and Balakrishnan (2023) evaluated the antioxidant and anticancer activities of *Lepidagathis pungens* and identified a few biomedical uses of a few significant species within this significant plant family.

The medicinal role and significance of the family can hardly be overemphasized. The present study highlights some key features of some important genera of the Acanthaceae family and also evaluates the antioxidant activity of acetone leaves extraction (50%) of 24 different species belonging to 13 genera of Acanthaceae, namely *Asystasia, Ballochia, Barleria, Blepharis, Crossandra, Dicliptera, Ecbolium, Hypoestes, Justicia*, *Megalochalmyas, Phaulopsis, Ruellia* and *Trichocalyx* which belongs to the family Acantheceae, which is being widely used as folk medicine.

MATERIALS AND METHODS

Plant materials

Leaves of different species belonging to Acanthaceae, namely: *Asystasia, Ballochia, Barleria, Blepharis, Crossandra, Dicliptera, Ecbolium, gymnostachyum, Hypoestes, Justicia, Megalochalmyas, Phaulopsis, Ruellia and Trichocalyx* were collected from different locations in Yemen (Figure 1). Plant material was identified by using the handbook of the Yemen flora (Wood, 1997) and Ethnoflora of the Soqotra Archipelago by Miller (2004) and the voucher specimens are kept at the herbarium, Universiti Kebangsaan Malaysia (UKMB).

Plant extraction

0.1 g powdered dried leaves were extracted using 10 mL of 50% acetone and homogenized using a homogenizer (T 250, IKA, Germany) at 24,000 r.p.m for 1 min. The extracts were centrifuged (MLX 210, Thermo-line, China) at 5000 ×*g* for 10 min. The supernatants were stored for assays.

Total Phenolic Content

The total phenolic compounds (TPC) of the plant leaf extracts were determined using the Folin-Ciocalteu method (McDonald *et al*., 2001). An amount of 0.4 mL of distilled water and 0.5 mL of dilutedFolin–Ciocalteu reagent were added to 100 µL of the extracts. The samples (sample extracts mixed with Folin–Ciocalteu reagent) were retained for 5 min and 1 mL of solution constituting 7.5 % of sodium carbonate (w/v) was added. The extracts were thoroughly mixed and incubated at room temperature for 2 h and the spectral absorbance was measured at 765 nm using a spectrophotometer (Epoch Biotek, USA). The standard curve of gallic acid (GA) is used to estimate TPC. TPC of the samples was determined and the amounts of each compound in different extracts were expressed in mg of GA equivalent per gram of dried sample (mg GAE/g of DW).

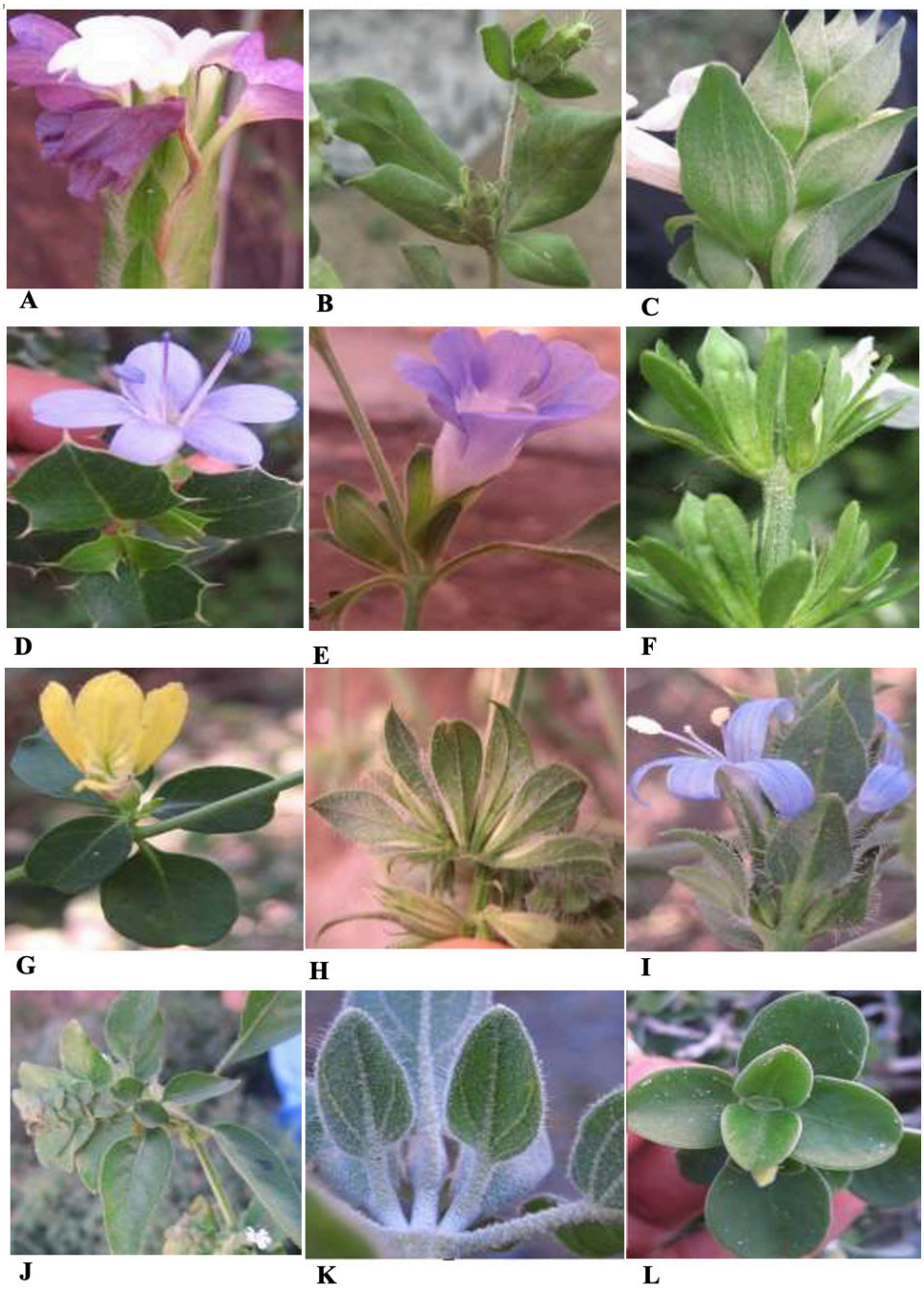

Fig. 1. Leaves morphology of different species; (A) *Crossandra johanninae*; (B) *Blepharis maderaspatensis*; (C) *Asystasia guttata*; (D) *Barleria aculeata;* (E) *B. ventricosa:* (F) *Justicia caerulea*; (G) *Justicia odora*; (H) *Dicliptera verticillata*; (I) *Megalochalmyas violacea*; (J) *Phaulopsis imbricata*; (K) *Ruellia grandiflora*; (L) *R. paulayana.*

Ferric Reducing Antioxidant Power

The Ferric Reducing Antioxidant Power (FRAP) of the extracts was determined after slight modification as recommended using Thaipong *et al*. (2006) method. Firstly, 300 mM acetate buffer FRAP reagent was freshly prepared using pH 3.6 (3.1 g sodium acetate trihydrate plus 16 mL glacial acid made up to 1:1 with distilled water); 10 mM 2,4,6-tris (2-pyridyl)-*s*-triazine (TPTZ) in 40 mM of HCl; and 20 mM of $FeCl_3 \cdot 6H_2O$ in a ratio of 10:1:1 and used as the working reagent. 1 mL FRAP reagent is added to 100 µL of the extracts, and the absorbance was recorded at 595 nm using a spectrophotometer (Epoch, Biotek, USA).

Determination of Free Radical – Scavenging Activity of extract

The stock solution of free radicals was made by dissolving 40 mg DPPH in 100 mL of methanol and stored at -20 °C. Before the experiment, 350 mL DPPH stock solution was thoroughly dissolved in 350 mL methanol to ensure the absorbance was around 0.70±0.01 at 516 nm (Epoch, Biotek, USA). 100 µL extracts mixed with 1 mL methanolic DPPH solution are kept for 2 h in the dark. The percentage of DPPH scavenging activity is determined using the following equation: DPPH scavenging activity (%) = $[(A_{blank} - A_{sample}) / A_{blank}] \times 100$, where A is the absorbance.

Statistical analysis

Triplicated statistical analysis was done for each antioxidant activity assay to check for their consistency and to determine their reproducibility. Different tests having different content were analyzed using their mean value plus or minus standard deviation of different antioxidant activities. Duncan's new multiple-range tests are used to determine the significant differences of the specimen at a significance level of $P<0.05$. The correlation statistics of the data used in this study were calculated based on Pearson's correlation coefficient (r).

RESULTS AND DISCUSSION

In this study, 50% of acetone is used with the leaf extracts of 24 species of Acanthaceae collected from different locations in Yemen and widely used as traditional medicine for different ailments. The antioxidant activities of different extracts were reported in this study specifically focusing on their ability to scavenge (DPPH) stable free radical, ferric reducing capability of the antioxidant power (FRAP), and the concentration of phenolic content. This study is the first report of antioxidant studies in species *Asystasia guttata, Ballochia amoena, Barleria aculeata, B. orbicularis, B. parviflora, B. ventricosa, Blepharis cayaniense, Dicliptera effusa, D. paniculata, D. verticillata, Ecbolium gymnostachyum, Hypoestes pubescens, H. triflora, J. heterocarpa, J. odora, Megalochalmyas violacea, Phaulopsis imbricata, Trichocalyx orbicular*, and *R. paulayana.*

Evaluation of radical scavenging activity (DPPH)

The result showed that the ability of DPPH radical scavenging is higher in *Barleria ventricosa* (88.58%), *Barleria prionitis* (85.5%) *Ruellia grandiflora* (83.8%), *Crossandra johanninae* (82.5%) and *Barleria orbicularis* (80%) compare to *Hypoestes pubescens* (58.2%) and *Hypoestes triflora* (58.5%) that present less active (Figure 2). This study agreed with previous study made by (Sangilimuthu *et al*., 2012) and showed the higher ability of DPPH radical scavenging in root extract of *Barleria noctiflora.*

Ferric reducing antioxidant power (FRAP)

The results were presented in mg corresponding to Trolox equivalents/g of dry extracts. The FRAP value for the 24 species presented those significant differences exist in the reducing powers of the plant leaves species. The acetone extracts of *Barleria prionitis* (72.2 mgTE/g FW), *Trichocalyx orbiculatus* (69.4 mg TE/g FW), and *Ruellia grandiflora* (60.69 mg TE/g FW) have the highest reducing ability of Fe (III) while the lowest reducing is found in *Ruellia paulayana* (2.35 mg TE/g FW) and *Hypoestes pubescens* (2.47 mg TE/g FW) respectively (Figure 2). DPPH, TRAP, and FRAP analysis polar extracts have better antioxidant activity with significant differences ($p<0.05$), in the vent (Khlifi *et al*., 2013) which agrees with what present in this study.

Total phenolic concentration (TPC)

The concentration of phenolic acid showed different values which was higher in *Blepharis cayaniense, Ruellia grandiflora* (>800 µg/mL of GA). The concentration of phenolic content was found within a specific range (400-600 µg/mL of GA) in *A. guttata, B. prionitis, B. orbicularis, B. ventricosa, B. parviflora, Dicliptera* species while *H. pubescens* and *R. paulayana* species showed the lowest concentration of phenolic acid (>90 µg/mL of GA). Based on this result, species such as *Blepharis cayaniense, Crossandra johanninae, Justicia caerulea*, *Ecbolium gymnostachyum,* and *Trichocalyx orbiculatus* were found to be potential sources of antioxidants (Figure 2). Previous studies by Giorgi

et al. (2005) and Scalzo *et al*. (2005) explained the differences in correlation value exhibited during antioxidant activity and TPC.

In this paper, a positive relationship is found between TPC and DPPH for acetone extracts with *R*=0.64 while TPC showed a lower value of correlation relationship with FRAP (0.453). However, the correlation value between DPPH and FRAP was 0.54.

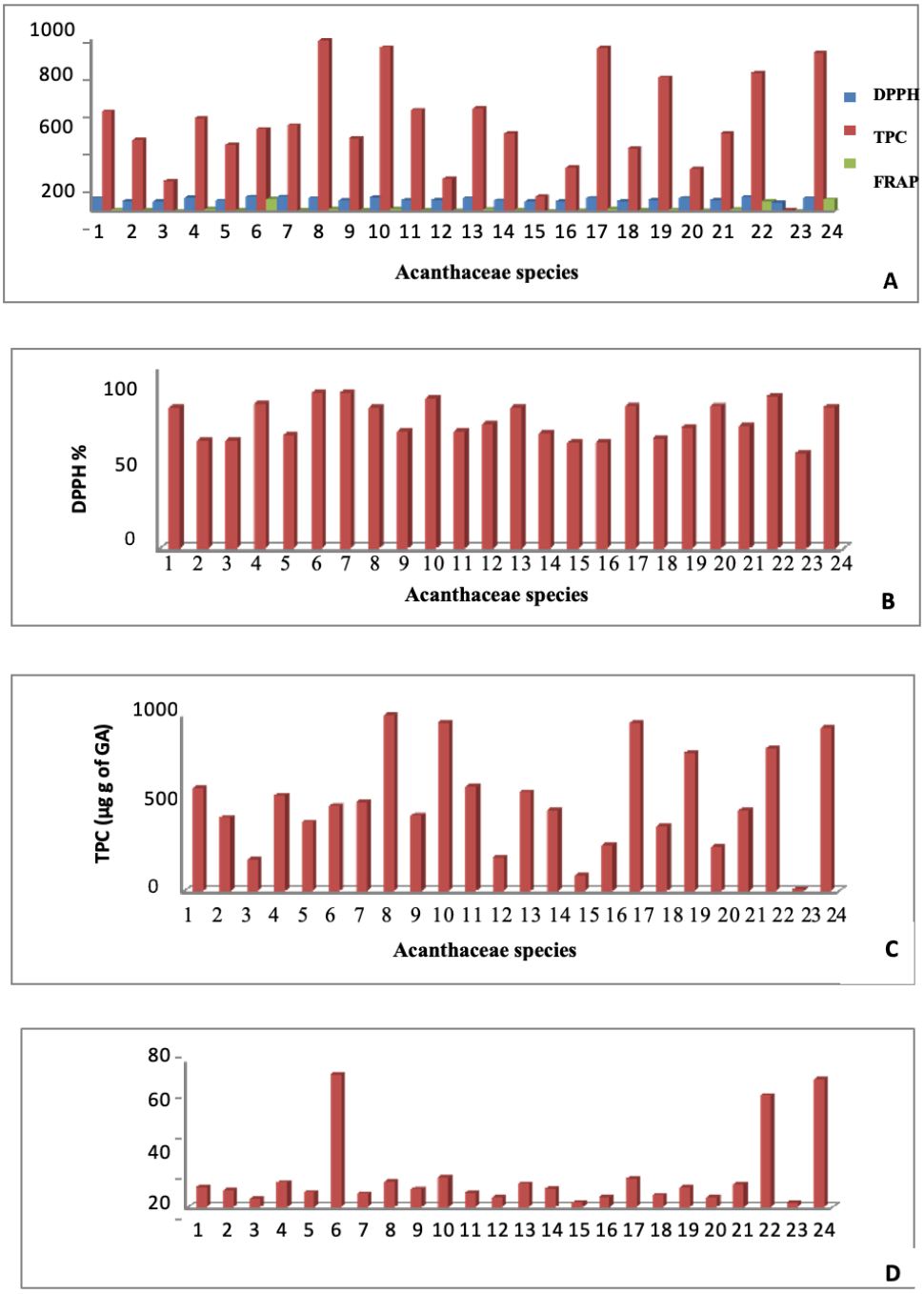

Fig. 2. A-D: Different antioxidant levels in DPPH, FRAP, and TPC & Numbers 1-24 referring to species of Acanthaceae

CONCLUSION

Results showed good antioxidant activity of the extracts with DPPH radical that exhibits good FRAP activities in phenol content when compared with the standard antioxidant compounds. The extracts containing organic solvent (Acetone 50%) shows significant antioxidant potential which reduces the DPPH radical formation and Fe (III) in addition to different concentration level of phenolic content. This finding contributes to the potential of leaf extracts of Acanthaceae to provide adequate protection against infections and degenerative diseases. In addition, the results also showed a good correlation between antioxidant activity and phenolic content using simple data to classify extracts from different species relative to their antioxidant potential.

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ETHICAL STATEMENT
Not applicable.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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