ANTIMICROBIAL AND ANTIOXIDANT POTENTIAL OF *IPOMOEA HEDERACEA*

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Abstract

Research on antimicrobial and anti-oxidant activity from plant sources is increasing due to positive effects on human health. However relatively little work has been done to investigate the antimicrobial and antioxidant effects of methanolic extracts from *Ipomoea hederacea.*

In the present study methanolic extracts of leaves, flowers, stem, seed and roots from *Ipomoea hederacea* were evaluated using an antimicrobial assay, the determination of total phenolic content, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical and the total antioxidant capacity.

Our results suggested that methanolic extracts exhibited high antimicrobial effects when tested against various bacterial and fungal strains (i.e. *B. subtilis, P. multocida, S. aureus, E. coli, A. niger, A. flavus, A. alternate* and *R. solani*). The total phenolic content showed the highest value for stem extract (131.11±1.22 mg/g), and the lowest for root extract (74.44±1.52 mg/g). The tested extracts were able to reduce the stable DPPH radical, reaching IC50 values from 83.14±1.02 µg/mL for stem extract to 123.32±1.83 µg/mL for root extract. A positive linear correlation was observed when total phenolic content was correlated with the total antioxidant capacity.

Our study sustains the fact that methanolic extracts from *Ipomoea hederacea* can be used as components in a range of phytochemical formulation due to their antimicrobial and antioxidant potential.

Rezumat

Cercetările asupra potenţialului antimicrobian şi antioxidant a unor specii vegetale se îndreaptă către efectele acestora asupra sănătăţii umane. În literatura de specialitate se regăsesc puţine studii ştiinţifice privind efectele antimicobiene şi antioxidante ale extractelor metanolice de *Ipomoea hederacea.*

În studiul de faţă, extractele metanolice din frunze, flori, tulpini şi rădacini de *Ipomoea hederacea* au fost evaluate în ceea ce priveşte testarea antimicrobiană, determinarea conţinutului fenolic total, a activităţii radicalilor liberi de 2,2-difenil-1-picrilhidrazil (DPPH), cât şi a capacităţii totale antioxidante.

Rezultatele noastre au arătat faptul că extractele metanolice prezintă efecte antimicrobiene crescute la testarea pe diferite tulpini bacteriene şi fungice (de exemplu *B. subtilis*, *P. multocida*, *Staphylococcus aureus*, *E. coli*, *A. niger*, *A. flavus*, *A. alternate* şi *R. solani*). Conţinutul fenolic total a prezentat cea mai mare valoare în extractul de tulpină (131,11±1,22 mg/g), iar cea mai mică valoare în extractul de rădăcină (74,44±1,52 mg/g). Extractele testate au fost capabile să reducă radicalul DPPH, atingând o valoare a IC_{50} de la 83,14±1,02 µg/mL pentru extractul de tulpină până la 123,32±1,83 µg/mL pentru extractul de rădăcină. A fost observată o corelaţie pozitivă când conţinutul fenolic a fost comparat cu capacitatea totală antioxidantă.

Studiul nostru susţine faptul că extractele metanolice de *Ipomoea hederacea* pot fi folosite într-o serie de compoziţii fitochimice datorită potenţialului antimicrobian şi antioxidant.

Keywords: *Ipomoea hederacea*, phenolic compounds, antimicrobial, antioxidant

Introduction

In recent years, there has been an increasing trend towards the exploration of safer and effective antioxidant and functional ingredients from natural dietary sources like vegetables [1]. The antimicrobial and antioxidant effects of vegetables like *Ipomoea hederacea* also known as ivy-leaved morning glory, is mainly due to the occurrence of phenolic compounds [2, 3].

However, few reports dealing with the obtaining of different methanolic extracts and further determination of the compounds with antimicrobial and antioxidant potential can be found in literature mainly due to the low efficiency (in terms of quantities) in their extraction that did not encourage further investigations of these useful and promising fractions [4, 5].

Biological screening includes cytotoxic, phytotoxic or an anthelmintic activity is an important part of the development of new research strategies from medicinal plants. In this respect, bioactive compounds isolated from different plants are known to be effective and versatile chemopreventive and antitumor agents in many experimental models of carcinogenesis [6]. However, how these biological compounds

from *Ipomoea hederacea* can interfere into both antimicrobial and antioxidant process is still under debate [7, 8].

In the present study, the antimicrobial and antioxidant potential of methanolic extracts from *Ipomoea hederacea* in terms of antimicrobial assay, determination of total phenolic content, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical and total antioxidant capacity were assessed.

Materials and Methods

Extraction

Ipomoea hederacea plants were collected from the local supermarkets and kept at -18^{0}C. The identification was carried out at the Department of Agronomy, Bahauddin Zakariya University from Pakistan. A specimen has been kept in the laboratory for future reference. The plant material (*Ipomoea hederacea* leaves, flowers, stem, seed and roots) was air-dried in shade and crushed to coarse power separately using a pestle and mortar. The plant material (0.5 kg each) was macerated in an aqueous methanolic mixture (80:20; v/v, 1L), at room temperature for fifteen days with occasional shaking. The process was repeated for three times with the same quantity of solvent mixture. The obtained extracts were combined, filtered under vacuum and concentrated under reduce pressure in a rotary evaporator (Q-214M2, Quimis, Brazil) using a warm water bath (Q-214M2, Quimis, Brazil) to obtain a thick gummy mass, which was further dried in a desiccator and stored in air-tight vial and prepared for further experiments.

Antimicrobial Assay

The minimum inhibitory concentration (MIC) of the plant extract was evaluated by a modified resazurin microtiter plate assay after the method of Rizwan et al. [9]. Briefly, a volume of 100 µL of each extract solution was transferred into the first row of the 96 well plates. Then, 50 µL of nutriewnt broth and Muller Hinton broth for bacterial and fungal strains (i.e. *B. subtilis, P. multocida, E. coli, S. aureus, A. niger, A. flavus, A. alternate*, *R. solani*) were added.

Two-fold serial dilutions were performed using a multichannel pipette such that each well had 50 µL of the test material in serially descending concentrations. To each well, 10 µL of resazurin indicator solution (prepared by dissolving 270 mg resazurin tablet in 40 mL of distilled water were added. Finally 10 µL of bacterial/fungal suspension were added to each well. Each plate was wrapped loosely with aluminium foil. Each plate had a set of controls: a column with antibiotics (i.e. Rifampicin 100 µL and Fluconazole 100 µL) as positive control, a column

with all solutions with the exception of the test samples, a column with all solutions with the exception of the bacterial/fungal solution adding 10 µL of broth instead and a column with respective solvents as a negative control. The plates were prepared in triplicate, and incubated at 37±0.1°C for 20-24 h and 28±0.3°C for 40-48 h for fungi and bacteria, respectively. The absorbance was measured at 620 nm for fungus and at 500 nm for bacteria. The growth was indicated by colour changes from purple to pink until colourless. The lowest concentrations at which colour change appear was considered as the MIC value [10].

Antioxidant Assays

Determination of Total Phenolic Content

Extract sample was dissolved with 10 mL of acidified methanol (i.e. 1 % formic acid). The extracts were kept at 4°C, at dark prior to further analysis. The content of phenolic compounds was determined using the Folin-Ciocalteu method, based on the reduction of phosphor wolframe-phospohomolybdate complex by phenolic compounds to a blue reaction product. About 1mL of sample was diluted in 60 mL of distilled water, and the 5 mL Folin-Ciocalteu reagent, previously diluted 2 times, were mixed. After 5-10 min a 15 mL of 20% solution of sodium carbonate was added, and the obtained solution was diluted to 100 mL. The prepared samples were kept for 2 h at room temperature and the absorbance was measured at 760 nm.

The data were calculated according to the standard curve of catechin (0.10-0.20 mg/mL) a part of the chemical family of flavonoids and they were expressed as catechin equivalents per gram of extracts (mg CAE/g) [1].

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity

The ability of the extract to scavenge DPPH radicals was assessed as described previously [11, 12]. Freshly prepared ethanolic DPPH (0.1 mM; 1 mL) solution was added to different concentrations of extract (from 20 to 200 µg/mL). After half an hour, the absorbance was recorded at 517 nm. Results were expressed as percentage inhibition:

$$Inhibition(\%) = \frac{A_0 - A_1}{A_0} x100 \quad (1)$$

where A_0 = absorbance of the control, and

A_1= absorbance of the extract.

The percentage inhibition was plotted against extract concentration in order to calculate IC_{50} values, which in our experiments, is the

concentration (µg/mL) of extract that causes 50% loss of DPPH activity. Results were compared with the positive control, ascorbic acid.

Total Antioxidant Capacity (TAC) determination

Trolox equivalent antioxidant capacity (TEAC) assay

The reaction of 7 mmol/L aqueous solution of 2,2'-azino-bis(3-ethylbenzothiazoline-6.sulphonic acid (ABTS) with 2.45 mmol/L potassium persulfate, and allowing the mixture to stand in the dark at room temperature for 12-16 h before use, obtained a stable stock solution of $ABTS^{+}$.

In the next step, an $ABTS^{+}$ working solution was obtained by the dilution in ethanol of the stock solution to an absorbance of 0.70±0.02 AU at 734 nm, verified by a Hewlett-Packard 8453 Diode Array spectrophotometer (HP, Waldbronn, Germany) and used as mobile phase in a flow-injection system. Results were expressed as micromoles of Trolox per gram of plant extract (µmol TE/g) [13].

Ferric reducing antioxidant power (FRAP) assay

The principle of the FRAP method is based on the reduction of a ferrictripyridyl triazine complex to it's ferrous coloured form in the presence of antioxidants. Briefly, the FRAP reagent contained 5 mL of a 2,4,6-tripyridyl-s-triazine solution in 40 mmol/L HCl plus 5 mL of $FeCl_3$ (20 mmol/L) and 50 mL of acetate buffer (0.3 mol/L and pH=3.6) and was prepared freshly and warmed at 37^0C. Aliquots of 100 µl sample were mixed with 3 mL FRAP reagent and the absorbance of reaction mixture at 593 nm was measured spectrophotometrically after incubation at 37^0C for 10 min. For the calibration curve, five concentrations of $FeSO_4.7H_2O$ (1000, 750, 500, 250, 125 µmol/L) were used and the absorbances were measured in similar conditions as the samples. The values were expressed as concentration of antioxidants having a ferric reducing ability equivalent to that of 1 mmol/L $FeSO_4$. Results were expressed as micromoles of Fe^{2+} equivalents per gram of plant extract (µmol Fe^{2+}/g) [14].

Total radical-trapping antioxidant parameter (TRAP) assay

The method is based on the protection provided by antioxidants on the fluorescence decay of R-phycoerythrin (R-PE, lag-phase) during a controlled peroxidation reaction. Briefly, 120 µL of diluted sample were added to 2.4 mL of phospahte buffer (pH=7.4), 375 µL of bidistiled water, 30 µL of diluted R-PE and 75 µL of 2,2'-azo-bis(2-amidinopropnae)-dihydrochloride; the reaction kinetics at 38°C were recorded for 45 min by a LS-55 luminescence spectrometer (Perkin Elmer, Wellesley, MA). TRAP values were calculated from the length of lag-phase due to the sample

compared with that of Trolox and expressed as micromoles of Trolox per gram of extract (μmol TE/g) [15].

Statistical analysis

All the aforementioned experiments were conducted in triplicate. Statistical comparisons were performed by one-way analysis of variance (ANOVA). In the case of the identification of statistical differences using ANOVA, the Student Newmal-Keuls test was used to compare the fractions using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA). Probability values $p \leq 0.05$ were considered to indicate significant differences.

Results and Discussion

Infectious diseases produced by fungi and bacteria are the main cause of morbidity and mortality for humans in both developing and developed countries. Since the discovery of synthetic antibiotics and their use, it was believed that infectious diseases will be eradicated. However their over-use has led to production of drug resistant strains [1] limiting their efficiency. In our study, methanolic extracts were able to block the bacteria resistance mechanism, contributing to disease control, treatment and eradication. Table I shows the MIC of methanolic extracts of *Ipomoea hederacea*.

Table I.

Minimal inhibitory concentration (MIC) of methanolic extracts of *Ipomoea hederacea* (mg/mL)

Strains tested	Stem	Root	Leaves	Flower	Rifampicin	Fluconazole
B. subtilis	14.22*±0.41	47.09*±0.33	21.52*±0.06	18.43*±0.11	11.17*±0.63	n.d
P. multocida	13.25*±0.39	43.34*±0.70	19.05*±0.48	16.01*±0.22	10.01*±0.22	n.d
S. aureus	8.04*±0.71	37.82*±0.02	14.35*±0.63	10.04*±0.19	6.88*±0.57	n.d
E. coli	89.39*±1.06	148.24*±1.66	113.08*±0.04	101.22*±1.75	72.06*±1.62	n.d
A. niger	111.08*±0.28	173.21*±1.52	138.91*±1.25	122.65*±1.43	n.d	101.33±0.28
A. flavus	12.08*±0.07	41.02±0.68	17.21*±0.23	14.11*±0.03	n.d	8.09±0.08
A. alternata	46.22*±1.13	94.04*±1.05	63.34*±10.8	54.60*±0.58	n.d	31.71±1.05
R. solani	16.68*±0.09	59.37*±0.98	37.52*±0.86	23.34*±0.66	n.d	9.11±0.44

Data are expressed as the mean ± standard deviation; *$p < 0.05$ (nd = not detected).

The analysed extracts exhibited high antimicrobial effects when tested against various bacterial and fungal strains like *B. subtilis, P. multocida, S. aureus, E. coli, A. niger, A. flavus, A. alternate* and *R. solani.*

Other reports showed that the highest MIC values were obtained for pineapple honey against *E. coli* and *P. aeruginosa* as well as acacia honey

against *E. coli* with 25% (w/v) MIC and 50% (w/v) minimal bacterial concentration values [16].

Prashanth and contributors [17] also reported methanolic extract of *Punica granatum* fruit ring to be active against all microorganisms tested in their study. Mathabe and contributors [18], showed that methanol, ethanol, acetone and water extracts obtained from *Punica granatum* were active and effective against the tested microorganism (*S. aureus*, *E. coli*, *S. typhi*, *V. cholera*, *S. dysenteriae*, *S. Sonnei*, *S. flexneri* and *S. boydii*) showing an inhibition zones of 12-31 mm. Although there are few studies concerning the antimicrobial and antibacterial potential of such medicinal plants, these studies are in accordance to our results, showing the same higher antimicrobial activity against different bacterial cultures tested [1, 16, 17, 18].

The total phenolic content as measured by Folin-Ciocâlteu method results are shown in Table II. The highest value was obtained for the stem extract (131.11±1.22 mg CAE/g), and the lowest was registered in the root extract (74.44±1.52 mg CAE/g).

Table II.

Antioxidant activity of plant extracts of *Ipomoea hederacea* expressed by total phenolic content

Antioxidant Assay	Stem	Leaves	Roots	Flower
Total phenolic content (mg CAE/g)	131.11* ± 1.22	90.13* ± 1.63	74.44* ± 1.52	105.26* ± 1.52
TEAC (µmol TE/g)	186.51* ± 2.06	152.70* ± 1.92	131.42* ± 1.66	174.22* ± 1.31
FRAP (µmol Fe^{++}/g)	301.09a ± 3.12	262.45* ± 2.12	236.29* ± 2.28	285.13* ±3.04
TRAP (µmol TE/g)	688.47* ± 3.09	624.33* ± 3.66	597.55*± 2.77	655.01* ± 2.70

CAE = catechin equivalent; TEAC = trolox equivalent antioxidant capacity; FRAP = ferric reducing antioxidant power; TRAP = total radical-trapping antioxidant parameter; TE = Trolox equivalent.
Data are expressed as the mean ± standard deviation; *p < 0.05 (nd = not detected).

Our results are partially agree with those found for other *Ipomoea* species. Phenolic compounds possess a wide spectrum of biological activities like anticancer, antioxidant and gene-modifying capacities [19, 20]. Interestingly, *Ipomoea hederacea* extracts showed also a high antioxidant capacity, reducing different types of free radicals. When excess amounts of free radicals cannot be counter balanced by human body defence system, these radicals attack biomolecules leading to a number of disorders. The tested extracts were able to reduce the stable DPPH radical, reaching IC_{50} values from 83.14±1.02 µg/mL for stem extract to 123.32±1.83 µg/mL for the root extract. The DPPH radical scavenging activity of the extracts is concentration dependent and a lower IC_{50} reflects a better protective potential. Our results are different from values obtained from other *Ipomoea* species [6]. Moreover, the effects of the compounds mentioned above and contained *Ipomoea hederacea* seeds could be attributed to the direct

implication of interleukins, which are known to have important modulatory effects on proinflammatory cytokines [21].

Interestingly, results obtained for TEAC, FRAP and TRAP assays are in agreement with other studies on *Ipomoea hederacea* seeds antioxidant capacity [7, 22].

A simple linear regression analysis was used to analyse the correlation between the TEAC values and total phenolic contents (Table II and Figure 1a).

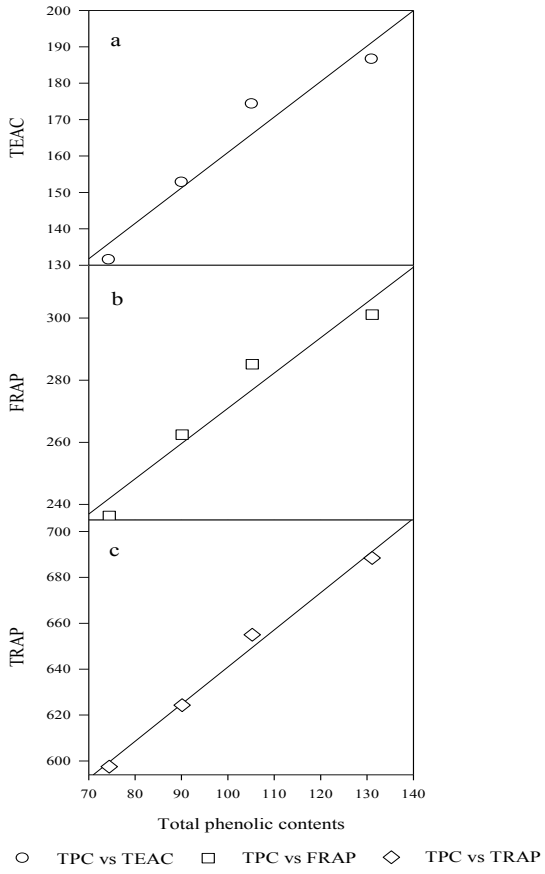

Figure 1.

Correlation between the total phenolic contents (TPC) and (a) Trolox equivalent antioxidant capacity (TEAC), (b) ferric reducing antioxidant power (FRAP) and (c) total radical-trapping antioxidant parameter (TRAP) assays.

A positive linear correlation was observed when the total phenolic content was correlated with all TEAC, FRAP and TRAP assays (Figure 1a, b and c). The regression equation Y = 63.56+ 0.97x was statistically

significant (F=29.54, p<0.0322, R^2=0.936). The results suggest that the methods used are generally consistent for evaluating antioxidant capacities. Similarly for correlation between FRAP values and total phenolic content (Figure 1b), the regression equation Y=157.47+1.14x was statistically significant (F=33.74, p<0.0283, R^2=0.94). Furthermore, for the correlation between TRAP values and total phenolic content, the obtained regression equation Y=479.15 +1.62x was similar with the correlation between FRAP values and total phenolic content (F=211.04, p<0.0047, R =0.99) (Figure 1c).

The free radicals scavenging activity of crude extracts varies among species and even in the same species collected from different places, considering the time of collection, extraction process, solvent used and method employed. The results indicate a positive linear correlation, and the total antioxidant capacity of *Ipomoea hederacea* can be attributed to its phenolic content.

Conclusions

The extracts exhibited high antimicrobial effects tested against *B. subtilis, P. multocida, S. aureus, E. coli, A. niger, A. flavus, A. alternate* and *R. solani*. The higher total phenolic content showed high values from stem extract in respect with root extract, being able to reduce the stable 2,2-diphenyl-1-picrylhydrazyl free radical. Furthermore, a positive linear correlation was observed between total antioxidant capacity and the total phenolic contents. The results showed that the leaves, flowers, stem, seed and roots extracts from *Ipomoea hederacea* showed high antimicrobial and antioxidant activities. Our study sustained the fact that ivy-leaved morning glory can be used in a range of phytochemical formulations due to its antimicrobial and antioxidant potential.

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