ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *IRIS NIGRICANS* METHANOLIC EXTRACTS CONTAINING PHENOLIC COMPOUNDS

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Abstract

The presence of natural antioxidant and antimicrobial in plants is well known. This paper reports the antioxidative and antimicrobial activities of methanolic *Iris nigricans* extracts. Methanolic extracts of *Iris nigricans* (rhizomes, leaves and flowers) were evaluated for their free radical scavenging activity using the DPPH radical assay. Reduction of DPPH radicals can be observed by the decrease in the absorbance at $\lambda_{max517nm}$. The rhizomes extract, leaves extract, flowers extract and ascorbic acid showed antioxidant activity with different IC_{50} values. The antimicrobial activity of rhizomes extract, leaves extract and flowers extract was done by using disc diffusion method against different species of bacteria and against Candida albicans.

Keywords: Methanolic extracts of *Iris nigricans,* antioxidant activity, ascorbic acid, DPPH, free–radical assay, antimicrobial activity (bacteria species and Candida albicans), disc diffusion method.

Introduction

Antioxidants have an essential role in body defense system against Reactive Oxygen Species (ROS). Natural antioxidants that are present in the food increase the resistance toward oxidative damages and they may have an essential impact on human health. Therefore, consumption of food that is containing phytochemical with potential antioxidant properties can decrease the danger of human diseases. Chain breaking antioxidants are highly

reactive with free radicals and form stable compounds that do not contribute to the oxidative chain reaction.(Akira Yamauchi, et al., 2012)

In other way an antioxidant can be defined as a molecule that is capable of slowing or preventing the oxidation of other molecules. Antioxidants are often reducing agents such as thiols or polyphenols. They are believed to play an important role in preventing the development of such chronic diseases as cancer, heart disease, stroke, Alzheimer's disease, Rheumatoid arthritis, and cataracts. (Swati M. Devare, et al., 2012)

Some plant families exhibit antimicrobial activities including antibacterial and antifungal. Helicobacter pylori is susceptible to a wide range of antimicrobial agents, including *Acacia nilotica*, *Datura stramonium*, Mangifera indica and *Eucalyptus globulis*. It has been found that aglycones inhibit the growth of H pylori, whereas glycosides are inactive. The presence of a methoxyl group at C-4' was also important, and its replacement with a hydroxyl group caused a significant decrease in the activity of the compound. (Lisette D' Souza, et al., 2010) The antifungal fatty acids naturally can insert themselves into the lipid bilayer of the fungal membranes and physically disrupt the membrane, leading to enhanced fluidity of the membrane. These elevations in membrane fluidity will cause a generalized disorganization of the cell membrane that leads to conformational changes in membrane proteins, the release of intracellular components, cytoplasmic disorder and eventually cell disintegration. Antifungal free fatty acids can be saturated or unsaturated and in general the antifungal activity of fatty acids increases with an increase in chain length. Most common side effects of antifungal agents are nephrotoxicity and ototoxicity. (Satish, S. et al., 2007)

Methodology

Antioxidant Activity (DPPH free radical scavenging activity) for methanolic extracts of Rhizomes, Leaves and Flowers of *Iris nigricans*.

A- Rizomes

Antioxidant activity of *iris nigricans* parts and the standard were assessed on the basis of the radical scavenging effect of the stable 1,1- Diphenyl-2-picryl hydrazyl (DPPH) free radical activity by modified method. (Ilyas Chikhi, et al., 2012, Ritesh Jain, et al., 2011)

The diluted working solutions of the test extracts were prepared in methanol. The stock solution of rhizomes extract (1000 mg/ml) was prepared by dissolving weighted amount of the crude extract in 99.8% methanol. The dilution samples from rhizomes stock solution (5, 10, 15, 20, 40, 60, 80, 100, 200, 400 µg/ml) were prepared. Ascorbic acid was used as a standard with these concentrations (0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 µg/ml). These concentrations were prepared from ascorbic acid stock solution (100 mg/ml) using suitable

dilution by applying the dilution calculator equation. DPPH (0.002%) was prepared in methanol and 2 ml of this solution was mixed with 2 ml of sample solution of each dilution and the same with standard solution (ascorbic acid) separately. These solution mixtures were kept in dark for 30 minutes and the absorbance was read at λ_{max517} nm. Methanol with DPPH solution (2 ml: 0.002% /ml) was used as blank. Methanol (4 ml) was used as control. The absorbance was recorded and % of inhibition was calculated using the formula given as the following:% of inhibition = (Absorbance of control – Absorbance of test sample / Absorbance of control) x 100 (Ilyas Chikhi, et al., 2012, Ritesh Jain, et al., 2011)

B- Leaves

The stock solution of leaves extract (1000 mg/ml) was prepared by dissolving weighted amount of the crude extract in 99.8% methanol. The dilution samples from leaves stock solution (5, 10, 15, 20, 40, 60, 80, 100, 200, 400 µg/ml) were prepared. Ascorbic acid was used as a standard with these concentrations (0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 µg/ml). These concentrations were prepared from ascorbic acid stock solution (100 mg/ml) using suitable dilution by applying the dilution calculator equation. DPPH (0.002%) was prepared in methanol and 2 ml of this solution was mixed with 2 ml of sample solution of each dilution and the same with standard solution (ascorbic acid) separately. These solution mixtures were kept in dark for 30 minutes and the absorbance was read at λ_{max517} nm. Methanol with DPPH solution (2 ml: 0.002% /ml) was used as blank. Methanol (4 ml) was used as control. The absorbance was recorded and % of inhibition was calculated as described in the previous section. (Ilyas Chikhi, et al., 2012, Ritesh Jain, et al., 2011)

C- Flowers

The stock solution of flowers extract (1000 mg/ml) was prepared by dissolving weighted amount of the crude extract in 99.8% methanol. The dilution samples from flowers stock solution (1, 2, 4, 5, 8, 10, 11, 12, 15, 20, 40, 60, 80, 100 µg/ml) were prepared. Ascorbic acid was used as a standard with these concentrations (0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 µg/ml). These concentrations were prepared from ascorbic acid stock solution (100 mg/ml) using suitable dilution by applying the dilution calculator equation. DPPH (0.002%) was prepared in methanol and 2 ml of this solution was mixed with 2 ml of sample solution of each dilution and the same with standard solution (ascorbic acid) separately. These solution mixtures were kept in dark for 30 minutes and the absorbance was read at λ_{max517} nm. Methanol with DPPH solution (2 ml: 0.002% /ml) was used as blank. Methanol (4 ml) was used as control. The absorbance was recorded and % of inhibition was calculated as described in the previous section. (Ilyas Chikhi, et al., 2012, Ritesh Jain, et al., 2011)

Antimicrobial Activity

A- Antibacterial Screening

Antibacterial screening is generally performed by disc diffusion method. About 20 ml quantities of nutrient agar were plated in petri dishes with 0.1 ml of a 10 dilution of each bacterial culture (*Staphylococcus aureus*, *Escherichia coli*, *Bacillis subtilis*, and *Klebsiella pneumoniae*). Then 1 ml of distilled water was added to different methanolic extracts of *Iris nigricans* (leaves, rhizomes, flowers) to give concentrations of 264 mg/ml, 646 mg/ml and 229 mg/ml consequently. Filter paper discs (5 mm in diameter) impregnated with various concentrations of each of the above mentioned plant extracts were placed on test organism-seeded plates.The activity was determined after 18 h of incubation at 37°C. (Mahmoud A. Al-Qudaha,et al., 2012)

The results are presented in table (1-2).

B- Antifungal Activity

The antifungal activities of methanolic extracts of rhizomes, leaves and flowers of plant were determined by disc diffusion method against Candida albicans at the concentrations of 264 mg/ml, 646 mg/ml and 229 mg/ml consequently. The filter paper discs (5mm) were immersed in that concentrations. Then 20 ml quantities of Sabouraud dextrose were added in petri dishes with spreading 0.1 ml dilution of Candida albicans culture and incubated at temperature 37^0C. The impregnated discs were inoculated on the surface of petri dishes. The activity was determined after 18 h of incubation at 37°C.(Mahmoud A. Al-Qudaha,et al., 2012)

The results are presented in tables (1-3).

Results and Discussion

Statistical analysis:

Samples were analyzed in triplicate and the results were given as Mean ± S.D.

DPPH radical scavenging assay

Methanolic extracts of *Iris nigricans* (rhizomes, leaves and flowers) were evaluated for their free radical scavenging activity using the DPPH radical assay. Reduction of DPPH radicals can be observed by the decrease in the absorbance at $\lambda_{max517nm}$. The rhizomes extract, leaves extract, flowers extract and ascorbic acid showed antioxidant activity with different IC_{50} values as shown in table (1-1) and Figure (1-1).

The results indicated that the antioxidant activity of flowers extract was higher than ascorbic acid and the other crude extracts. The phytochemical analysis of different parts of *Iris nigricans* (rhizomes, leaves and flowers) extracts indicated the presence of major

phytocompounds as phenolic compounds (xanthones and flavonoids) and acids, which might be responsible for the antioxidant activity. (Livan Delgado Roche, et al., 2012)

DPPH_ is a stable nitrogen-centred free radical the color of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron-donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers. In order to assess the radical scavenging potential of the Iris (flowers, leaves and rhizomes) extracts, their reactivity towards the stable free radical DPPH was measured.DPPH is one of the chemical compounds that possess a proton free radical and it shows a maximum absorption at $\lambda_{max517nm}$ because of its bright purple colour.When DPPH encounters proton radical, its purple colour fades rapidly and this scavenging action forms the basic mechanism for measuring antioxidant activity.

From table (1-1), we observe that the flowers extract of *Iris nigricans* have the highest radical scavenging activitywith the lowest IC_{50} value of 2.549 µg/ml followed by the leaves extracts with an IC_{50} value of 26.436 µg/ml.

While the rhizomes extracts have the lowest radical scavenging activity with an IC_{50} value of 40.064 µg /ml.

Regarding free radical scavenging activity it was observed that flowers extracts showed a significant dose dependent inhibition of DPPH radical scavenging activity compared to leaves and rhizomes extracts. These flowers extracts exhibited a noticeable antioxidant effect at low concentrations (Table 1-1). This suggests that extracts contain compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity.

According to these results, there is a relationship between chemical composition and antioxidant activity. Moreover, as reported in literature data, the antioxidant activity of flowers extracts could be attributed to phenolic compounds, flavonoids and saponins.

The polyphenol compounds play a wide range of biological effects including antibacterial, anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, anticarcinogenic and cardioprotective and vasodilatory effects. These functions have been attributed to their antioxidant activity by several mechanisms such as free radical scavengers, reducing agents, complexers of pro-oxidant metals, quenchers of the formation of singlet oxygen and stimulating the antioxidative defence enzymes activities.These mechanisms will be led by two types of reactions: hydrogen atom transfer and single electron transfer. Flavonoids have been shown to exhibit their actions through effects on membrane permeability and by inhibition of membrane-bound enzymes such as the ATPase and

phospholipase A2and this property may explain the mechanisms of antioxidative action of *Iris nigricans.*

Table 1-1. Percentage of inhibitions of *Iris nigricans* extracts (flowers, leaves and rhizomes) at different concentrations using the DPPH radical assay.

Test compound (Methanolic extract) Conc.(µg/ml)	**% of Inhibitions**					
	5	10	20	60	100	IC_{50}
Flowers	74.900	93.100	98.900	99.544	99.848	2.549
Leaves	20.193	26.654	57.629	87.815	96.148	26.436
Rhizomes	9.666	15.817	35.325	85.764	95.782	40.064
Ascorbic acid	90.220	97.620	-	-	-	1.698

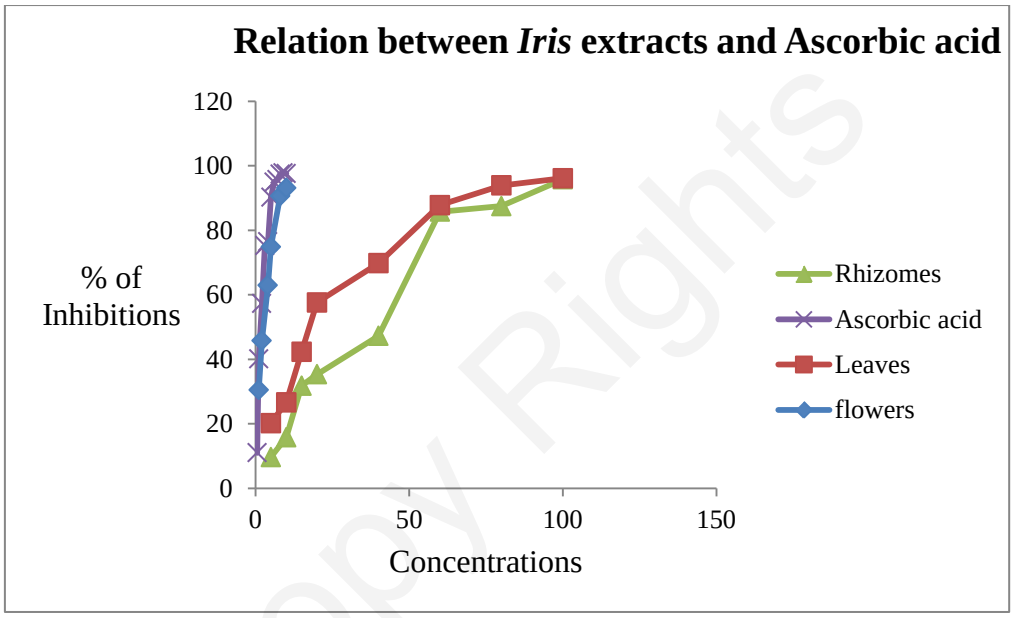

Figure 1-1: DPPH free radical scavenging activity of standard ascorbic acid and methanolic plant extracts.

Antimicrobial Study

Preliminary antibacterial and antifungal activities of methanolic extracts of *Iris nigricans* (rhizomes, leaves and flowers) were evaluated and presented in the following sections.

Antibacterial Screening

Antibacterial screening is generally performed by disc diffusion method for different methanolic extracts of *Iris nigricans* (leaves, rhizomes, flowers) in concentrations of 264mg/ml, 646mg/ml and 229mg/ml consequently. (Mahmoud A. Al-Qudaha,et al., 2012). The results are illustrated in table 1-2.

According to the results of antibacterial activity of different parts of *Iris nigricans*, leaves and rhizomes extracts exhibited antibacterial activity. These activities may be

attributed to the presence of phenolic components (flavonoids and xanthones) which can disrupt the cell membranes or inhibit the synthesis of certain enzymes.

Table 1-2. Antibacterial activity of different parts of *Iris nigricans*.

Test Microorganisms	Inhibition zones of test microorganisms (mm)		
	Leave Extract	**Rhizome Extract**	**Flower Extract**
	264mg/ml	**646mg/ml**	**229mg/ml**
Gram- positive			
Bacillis subtilis	10	7	Not clear
Staphylococcus aureus	8	6	Not clear
Gram-negative			
Escherichia coli	9	6	Not clear
Klebsiella pneumoniae	7	5	Not clear

Antifungal Activity

The antifungal activity of methanolic extracts of *Iris nigricans* (rhizomes, leaves and flowers) are presented in table (1-3), leaves extract shows stronger antifungal activity than rhizomes and flowers extracts. The inhibition zone of leaves extract against Candida albicans was 15 mm while the inhibition zone of the well known antifungal agent nystatin that was 17 mm. Therefore, the antifungal activity of leaves extract was comparable to that of nystatin. The antifungal fatty acids naturally can insert themselves into the lipid bilayer of the fungal membranes and physically disrupt the membrane, leading to enhanced fluidity of the membrane. These elevations in membrane fluidity will cause a generalized disorganization of the cell membrane that leads to conformational changes in membrane proteins, the release of intracellular components, cytoplasmic disorder and eventually cell disintegration. Antifungal free fatty acids can be saturated or unsaturated and in general the antifungal activity of fatty acids increases with an increase in chain length. (Satish, S. et al., 2007)

According to the results of antifungal activity of different parts of *Iris nigricans*, undecylenic acid ($CH_2CH(CH_2)_8COOH$) is a natural fungicide and is FDA approved in over-the-counter medications for skin disorders.The mechanisms of action appear to be

interference with fatty acid biosynthesis, which can inhibit germ tube (hyphae) formation. Medium-chain fatty acids have also been shown to disrupt the pH of the cell cytoplasm by being proton carriers, which interferes with viral replication mechanisms in infected cells. (Xing-Cong Li, et al., 2008)

Table 1-3. Antifungal activity of different parts of *Iris nigricans*.

Test Microorganisms	Inhibition zones of test microorganisms (mm)			
	Leaves Extract 264mg/disc	**Rhizomes Extract 646mg/disc**	**Flowers Extract 229mg/disc**	**Nystatin Reference antibiotic 100mg/disc**
Candida albicans	15	10	10	17

Conclusion

- ❖ No previous studies on leaves and flowers of *Iris nigricans* grown in Jordan.
- ❖ The methanolic extracts of different parts of *Iris nigricans* (rhizomes, leaves and flowers) possess antioxidant activity which related to the phenolic compounds (flavonoids, xanthones and phenolic acids).
- ❖ The methanolic extracts of different parts of *Iris nigricans* (rhizomes, leaves and flowers) possess antifungal activity this may be due to undecylenic acid.

References:

Akira Yamauchi, Akitoyo Ichinose, Chikage Kawai and Futoshi Kuribayashi, The phagocyte NADPH oxidase and bacterial infections, Kawasaki Medical Journal, 2012, Volume 3, No.81, Pages 11－18.

Ilyas Chikhi, Hocine Allali and Mohamed El Amine Dib, Free radical scavenging and antibacterial activity of essential oil and solvent extracts of *Iris planifolia* (Mill) from Algeria, Journal of Medicinal Plants Research, 2012, Volume 6, No.10, Pages 1961-1968.

Lisette D' Souza, Solimabi Wahidulla and Prabha Devi, Antibacterial phenolics from the mangrove *Lumnitzera racemoza*, Indian Journal of Marine Sciences, 2010, Volume 39, No.2, Pages 294 – 298.

Livan Delgado Roche and Ángela Fraga Pérez, Protective effects of *Mangifera indica L.* extract against lipofundin-induced oxidative stress in rats, Pharmaceutical Crops, 2012, Volume 3, Pages 94-98.

Mahmoud A. Al-Qudaha, Riyadh Muhaidat, Ibrahim N. Trawenh, Hala I. Al Jaber, Mousa H. Abu Zarga and Sultan T. Abu orabi, Volatile Constituents of Leaves and Bulbs of

Gynandriris Sisyrinchium and their Antimicrobial Activities, Jordan Journal of Chemistry, 2012, Volume 7, No.3, Pages 287-295.

Ritesh Jain and Sanmati Kumar Jain, Total phenolic contents and antioxidant activities of some selected anticancer medicinal plants from Chhattisgarh State, India., Pharmacologyonline, 2011, Volume 2, Pages 755-762.

Satish, S., Mohana, D.C., Ranhavendra, M.P. and Raveesha, K.A., Antifungal activity of some plant extracts against important seed borne pathogens of *Aspergillus* sp. Journal of Agricultural Technology, 2007, Volume 3, No.1, Pages 109-119.

Swati M. Devare, Chandrakant D. Shendkar, Pranav S. Tambe, Manas K. Santra, N.R.Deshpande and J.P.Salvekar, Antioxidant potential of Morinda Pubescence leaves, International Journal of ChemTech Research, 2012, Volume 4, No.4, Pages 1339-1342.

Xing-Cong Li,Melissa R. Jacob, Shabana I. Khan, M. Khalid Ashfaq, K. Suresh Babu, Ameeta K. Agarwal, Hala N. ElSohly, Susan P. Manly and Alice M. Clark, Potent *in vitro* antifungal activities of naturally occurring acetylenic acids, Antimicrobial Agents Chemotherapy, 2008, Volume 52, No.7, Pages 2442–2448