



PHYTOCHEMICAL STUDIES AND ANTIOXIDANT ACTIVITY OF LEAVES EXTRACTS OF *Corchorus olerius* L. (MOLOKHIA)

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ABSTRACT: The aim of this study was to investigate the antioxidant activity and phytochemical analysis of the leaves extracts of *Corchorus olerius*. The phytochemical screening was carried on the leaves extracts of molokhia, revealed the presence of some active ingredients such as, alkaloids, tannins, saponins, phenols, glycosides, steroids, terpenoids and flavonoids. The ethanolic leaves extracts were also evaluated for their total phenolic, total alkaloid, total flavonoids, saponins, tannins, ascorbic acid and antioxidant activity using 2,2-diphenyl-2-picrylhydrazyl (DPPH), radical scavenging assay. The results of the present study showed that the ethanolic leaves extracts of molokhia, which contains high amount of phytochemical compounds exhibited the greater antioxidant activity than petroleum ether and aqueous extracts. The high scavenging property may be due to hydroxyl groups existing in the phenolic compounds. Ethanol extract contained the total phenol of 9.35 and tannins of 6.01 as mg of gallic acid equivalents (GAE), alkaloids of 28.51 as mg of atropine equivalents (AE) and flavonoids of 13.84 as mg of quercetin equivalents (QE).

Key words: *Corchorus olerius*, Molokhia, DPPH, phytochemical screening, ethanolic extract phenolic compounds, antioxidant activity.

INTRODUCTION

Oxidation is a process that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, and in turn, these radicals can start chain reactions that damage cells. Free radicals can be generated in biological systems through a variety of processes and in order to have significant activity as a free radical, a molecule must have an unpaired electron and sufficient redox potential (Yen and Chen, 1995).

The reactive oxygen species are oxygen derived free radicals such as superoxide anion ($O_2^{\bullet-}$), hydroxyl (OH^{\bullet}), hydroperoxyl (OOH^{\bullet}), peroxy (ROO^{\bullet}) and alkoxy (RO^{\bullet}) radicals and non free radicals such as hydrogen peroxide (H_2O_2), hypochlorous acid ($HOCl$), ozone (O_3) and singlet oxygen (O_2) (Halliwell and Gutteridge, 1999). It can be formed in living organisms by both endogenously (respiration,

peroxisomes stimulation of polymorphonuclear leucocytes and macrophages) and exogenously (ionizing radiation, tobacco smoke, pollutants, pesticides and organic solvents) (Irshad and Chaudhuri, 2002). An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule (Yamagishi and Matsui, 2011). The characteristic feature of an antioxidant is ability to scavenge the free radicals due to their redox hydrogen donors and singlet oxygen quencher (Anokwuru *et al.*, 2011; Wu *et al.*, 2011). The free radicals can be scavenged by the natural (plants) and synthetic (butylated hydroxyl toluene, butylated hydroxyl anisol and tetra butyl hydro quinone) antioxidants (Mbaebe *et al.*, 2012). But the usages of these synthetic antioxidants are now replaced because the natural antioxidants could be considered as safer without any side effects (Meenakshi *et al.*, 2011). In recent decades, many researchers are interested in medicinal plants for evaluation of

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antioxidant phytochemicals such as phenols, flavonoids and tannins, which have received more attention for their potential role in prevention of human diseases (Upadhyay *et al.*, 2010).

Plants play a vital role in the maintenance of human health (Moerman, 1996). Plants are endowed with various phytochemical molecules such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity (Zheng and Wang 2001 and Cai *et al.*, 2003). Studies have shown that many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities (Rice-Evans *et al.*, 1995 and Sala *et al.*, 2002). The ingestion of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing (Ashokkumar *et al.*, 2008; Veerapur *et al.*, 2009), and in recent years, there has been a worldwide trend towards the use of the natural phytochemicals present in berry crops, teas, herbs, oilseeds, beans, fruits and vegetables (Kitts *et al.*, 2000; Wang and Jiao, 2000; Muselik *et al.*, 2007). In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Krishnaraju *et al.*, 2005). Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections (Baladrin *et al.*, 1985). Vegetables are the fresh and edible portions of herbaceous plants which can be eaten raw or cooked (Fayemi, 1999 ; Dhellot *et al.*, 2006). They contain valuable food ingredients which can be successfully utilized to build up and repair body tissues. They are valuable in maintaining alkaline reserve of the body and are valued mainly for their high carbohydrates, vitamins and mineral contents. Vegetables may be edible roots, stem, leaves, fruits or seeds. Each group contributes to diet in its own way (Robinson, 1990)

Corchorus olitorius Linn. is an annual herb with slender stems. *C. olitorius* (Jute) is an important green leafy vegetable in many tropical

areas including Egypt, it is cultivated to provide bark for the production of fibres (Jute) and its mucilaginous leaves are used in food as a vegetable (Abou Zeid, 2002). The present study was carried out for qualitative and quantitative phytochemical analyses and *in vitro* antioxidant activities of leaves of *Corchorus olitorius*.

MATERIALS AND METHODS

Chemicals and Reagents

Chemicals used in this study were of analytical grade and purchased from El-Gomhoria Co. All solutions, including freshly prepared doubled distilled water. Stock solutions of the test extracts were prepared in ethanol.

Plant Material

Plant material was collected from the hyper market in Zagazig city on July 2016. Plant samples were dried under shade at room temperature and then ground in a mortar into fine powder. Then stored in airtight containers at room temperature.

Extract Preparation

The leaves were dried under shade and powdered in a mechanical grinder. The powdered material (200g) was extracted successively in Ethanol (70%), Petroleum Ether and distilled water by cold percolation method using Soxhlet apparatus at 55°C for 18 hr. The extracts were concentrated *in vacuo* and kept in a vacuum desiccators for complete removal of solvent and weighed.

Quantitative Phytochemical Analyses

The phytochemicals which are present in the solvent extracts of leaves of *Corchorus olitorius* L. were determined and quantified by standard procedures. (Trease and Evans, 1978; Khandelwal, 2006; Sofowora, 2008).

Determination of total alkaloids

A total of 200 ml of 20% acetic acid was added to 5 g of leaves powder taken in a separate 250 ml beaker and covered to stand for 4 hr. This mixture containing solution was filtered and the volume was reduced to one quarter using water bath. To this sample, concentrated NH₄OH was added drop-wise until

the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed (Harborne, 1973). The percentage of total alkaloid content was calculated as follows:

Percentage of total alkaloids = $\frac{\text{Weight of residue}}{\text{weight of sample taken}} \times 100$

Determination of total phenolic compounds

The total phenolics content of *C. olitorius* was estimated using Folin-Ciocalteu reagent by the method of Siddhuraju and Becker (2003). About 20 μg of leaves extract was taken separately and it was made up to 1ml with distilled water. Then 500 μl of diluted Folin-phenol reagent (1:1 ratio with water) and 2.5ml of sodium carbonate Na_2CO_3 (20%) were added. The mixture was shaken well and incubated in dark condition for 40 min for the development of colour. After incubation, the absorbance was measured at 725nm. A calibration curve of gallic acid was constructed and linearity was obtained in the range of 10-50 $\mu\text{g}/\text{ml}$. The total phenolics content in the leaves extract was expressed as mg of gallic acid equivalent (mg GAE/100g extract) by using the standard curve.

Total flavonoids content

The total flavonoids content was estimated using the procedure described by Kumaran and Karunakaran (2006). A total of 1ml of leaves extracts were diluted with 200 μl of distilled water separately followed by the addition of 150 μl of sodium nitrite (5%) solution. This mixture was incubated for 5min and then 150 μl of AlCl_3 (10%) solution was added and allowed to stand for 6min. Then 2 ml of NaOH (4%) solution was added and made up to 5 ml with distilled water. The mixture was shaken well and left for 15min at room temperature. The absorbance was measured at 510 nm. Appearance of pink colour showed the presence of flavonoids content. The total flavonoids content was expressed as rutin equivalent mg RE/100g extract on a dry weight basis using the standard curve.

Estimation of tannins content

Tannins content of *C. olitorius* was estimated by the method of (Siddhuraju and Manian,

2007). A total of 500 μl of the leaves extract was taken in test tube separately and treated with 100 mg of polyvinyl polypyrrolidone and 500 μl of distilled water. This solution was incubated at 4 °C for 4hr. Then the sample was centrifuged at 10000 $\times g$ for 5min and 20 μl of the supernatant was taken. This supernatant has only simple phenolics free of tannins (the tannins would have been precipitated along with the polyvinyl polypyrrolidone). The phenolics content of the supernatant was measured at 725 nm and expressed as the content of free phenolics on a dry matter basis. From the above results, the tannins content of the extract was calculated as follows:

Tannins (mg GAE/100g extract) = $\frac{\text{Total phenolics (mg GAE/100g extract)} - \text{Free phenolics (mg GAE/100g extract)}}{\text{mg GAE/100g extract}}$

Estimation of total saponins content

Estimation of total saponins content was determined based on vanillin-sulphuric acid colorimetric reaction with some modifications (Makkar *et al.*, 2007). About 50 μL of leaves extract was added to 250 μl of distilled water. To this, about 250 μl of vanillin reagent (800 mg of vanillin in 10 ml of 99.5% ethanol) was added. Then 2.5 ml of 72% sulphuric acid was added and it was mixed well. This solution was kept in a water bath at 60°C for 10 min. After 10min, it was cooled in ice cold water and the absorbance was read at 544nm. The values were expressed as diosgenin equivalents (mg DE/100g extract) derived from a standard curve.

Ascorbic acid (vitamin C)

Ascorbic acid determination was done according to (Klein and Perry, 1982). About 10 mg of dried leaves powder was re-extracted with 10 ml of 1% metaphosphoric acid. They were allowed to stand for 45min at laboratory temperature and filtered through Whatman No. 4 filter paper. About 1ml of filtrate was taken and it was mixed with 9 ml of 50 μmol 2,6-dichloroindophenol sodium salt hydrate and the absorbance was measured with 30min at 515 nm. Ascorbic acid content was calculated on the basis of the calibration curve of authentic L-ascorbic acid and the results were expressed as

mg of ascorbic acid equivalent (mg AE/100g extract).

Antioxidative Activity

The antioxidant activity of Molohkia (Leaves) on the basis of the scavenging activity of the stable (DPPH) free radical was determined according to the method described by Brand-Williams *et al.* (1995) with slight modification. The following concentrations of ethanol extract were prepared 20, 40, 60, 80 and 100 µg/ml. All solutions were prepared with methanol. 5 ml of each prepared concentration was mixed with 0.5 ml of 1mM DPPH solution in methanol. The test tubes were incubated for 30 min at room temperature and the absorbance measured at 517nm. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. Vitamin C (0.1 mg/ml) was used as a standard and the same concentrations were prepared as the test solutions. The difference in absorbance between the test and the control (DPPH in methanol) was calculated and expressed as (%) scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated by using the following equation.

$$\text{Scavenging effect (\%)} = (1 - A_s/A_c) \times 100$$

As is the absorbance of the sample at t = 0 min. Ac is the absorbance of the control at t = 30 min.

Statistical Analysis

Statistical analysis was carried out by One-way analysis of variance (ANOVA) test using a statistical package program (SPSS 10.0) and the significance of the difference between means was determined by Duncan's multiple range test at ($P < 0.05$) significant level. Analysis was carried out in triplicate and mean \pm SD of three parallel measurements described by (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The Effect of Different Solvents on the Yields of Molohkia Leaf Extracts

The significant variation in the yields of Molohkia leaf extracts was shown using various fraction solvents. The yields of extracts using aqueous, petroleum ether and ethanol in

Molohkia were 21.20g, 20.35g and 22.13g, respectively. The variation in yield may be due to the polarity of the solvents used in the extraction process. The results on the qualitative analysis of phytochemical constituents of different extracts of molohkia leaves are shown in Table 1.

Quantitative Phytochemical Analysis

Total phenolics content of various extracts of leaves of *C. olitorius* was varying widely between 5.28 to 9.35mg GAE/100g extract (Table 2). Ethanol extract was demonstrating higher total phenolics content (9.35 GAE/100g extract) than that of the other solvent extracts. The total flavonoids content was high in petroleum ether leaf extract (17.13 mg RE/100g extract) followed by ethanol and water extracts (13.84 and 11.68 mg RE/100g extract, respectively) (Table 2). The gravimetric analysis for total alkaloid contents in leaf of *C. olitorius* exhibited that higher alkaloid contents were present in leaf powder (28.51mg/100g sample). The tannins content of the various extract of leaves of *C. olitorius* was determined to be ranged between 4.17 and 6.01mg GAE/100g extract (Table 2). Among the solvents used the ethanolic leaf and petroleum ether extracts were registered high amount of tannins, 4.17 and 6.01 mg GAE/100 g extract, respectively. The total saponins contents of both leaves of *C. olitorius* were ranging between 20.73 and 16.69 mg DE/100 g extract across the various solvent extracts studied (Table 2).

Among the used solvents, the ethanol has drawn high amount of ascorbic acid content, 62.44 AE/100g extracts from leaves. On the other hand, water leaves extract extract contained lower amount of ascorbic acid (42.00 mg AE/100g extract) (Table 2).

Free Radical and Antioxidative Activity

Fig. 1 shows the results of the free radical (DPPH) scavenging activity in (% inhibition). The result revealed that the ethanolic fraction of Molohkia exhibited the highest radical scavenging activity with 71.42 ± 0.04 followed by its aqueous extract with 68.87 ± 0.09 and petroleum ether extract with 64.76 ± 0.06 . (Fig. 1). In overall comparison of different extracts the ethanolic extract of Molohkia show

the highest scavenging activity followed by the aqueous and then petroleum ether. Methanol and

Table 1. Results on the qualitative analysis of phytochemical constituents of different extracts of molohkia leaves

Phytochemical	Petroleum ether	Ethanol	Aqueous extract
Amino acids	–	++	++
Anthraquinones	–	+	+
Carbohydrates	–	++	++
Flavonoids	+	+	++
Glycosides	+	+	+
Gum and Mucilages	–	++	+
Phenols	+	+	+
Proteins	–	+	–
Reducing sugar	–	++	+++
Resins	+	+	+
Saponins	+	+	–
Steroid	+	++	+
Tannins	+	+	+
Terpenoids	+	+	+
Alkaloids	+	+	+

+ = The presence of phytochemical constituents. – = The absence of phytochemical constituents.

Table 2. Total phenolics, total alkaloids, total flavonoids, tannins, saponins and vitamin C contents of various alcoholic and aqueous extracts of leaves of *C. olitorius* (Molokhia)

Sample	Total phenolics ¹	Total flavonoids ²	Total alkaloids ³	Tannins ¹	Vitamin C ³	Saponins ⁴	IC ₅₀ Value (µg/ml)
Petroleum ether	5.66±0.02	17.13±0.05	20.0±0.17	4.17±0.01	15.30±0.21	20.73±0.06	0.0086
Ethanol	9.35±0.03	13.84±0.06	28.51±0.16	6.01±0.09	62.44±0.31	19.46±0.02	0.0054
Aqueous	5.28±0.04	11.68±0.03	23.4±0.10	5.07±0.01	42.00±0.05	16.96±0.03	0.0064

Values were performed in triplicates and represented as mean ± SD.

¹: mg GAE/100g extract, ²: mg RE/100g extract, ³: mg AE/100g extract, ⁴: mg DE/100g extract.

Mean values followed by different superscript in a column are significantly different ($P < 0.05$)

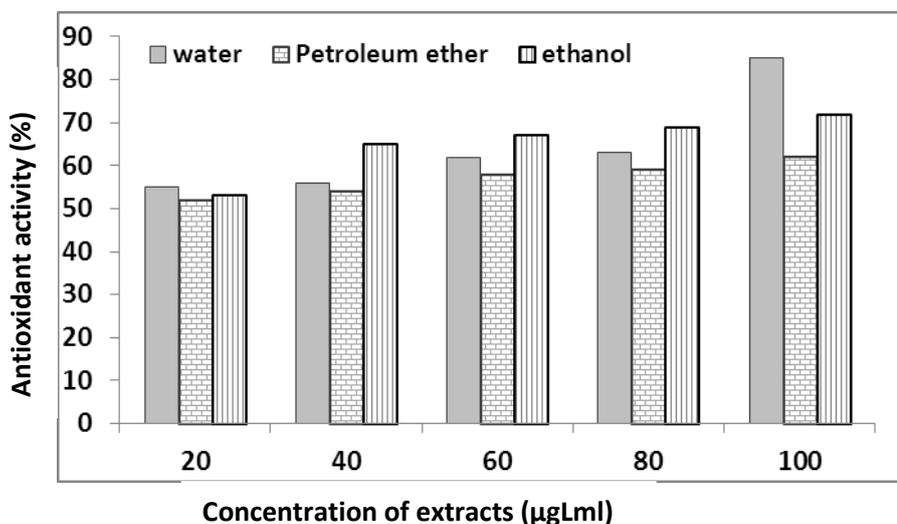


Fig. 1. Antioxidant activities of Molohkia in different extracts

ethanol has been proven as effective solvent to extract phenolic compounds (Siddhuraju and Becker, 2003). In the present study, the values of ethanolic and aqueous extracts were higher than petroleum Ether. Among used solvents in this study, ethanol has showed the best effectiveness extracting of phenolic components. Ethanol is preferred for the extraction of antioxidant compounds mainly because its lowers toxicity (Karadeniz *et al.*, 2005). Fig. 1. Shows the antioxidant activities of Molohkia in different solvents.

Antioxidant Activity

Therefore, we assume that DPPH free radical scavenging activity is related to the presence of bioactive compounds, such as phenolic compounds in extracts. The total phenolic content varied significantly between the different extracts of Molohkia. The highest concentration of total phenolic compounds were 492µg/mg presented in the ethanolic extract, followed by petroleum ether (412µg/mg) and aqueous extract (305µg/mg) of Molohkia.

IC₅₀ value

IC₅₀ value is defined as the concentration of substrate that causes 50% loss of the DPPH activity and was calculated by linear regression

mentioned of plots of the percentage of antiradical activity against the concentration of the tested compounds. Results in Table 2 reports IC₅₀ of extracts of Molohkia showed lower IC₅₀ value, however ethanolic extract of Molohkia being the lowest. The ethanolic extract of Molohkia exhibited significant activity with low IC₅₀ value. The antioxidant activity of Molohkia extracts rise with the rising of polyphenol content of the extract. A linear relationship between the reciprocal of IC₅₀ value and the total polyphenol content was observed in this study, indicating that increasing the polyphenol content strengthens the antioxidant activity. This finding is similar to that reported by (Katsube *et al.*, 2004).

Generally, majority of the secondary metabolites studied and ascorbic acid in leaves of *C. olerius* have present with higher amount in ethanolic extract than that of the other alcoholic and aqueous solvents. However, flavonoids and saponins were rich in petroleum ether extracts. It is explained that the polarity level and species nature are playing major role in extracting the secondary metabolites.

Conclusion

Ethanolic extract of *Corchorus olerius* L (Molohkia) exhibits the inimitable inhibition of

the free radical scavenging activity when compared with the other solvent extracts. Potentially we recommended that ethanol solvent extract is the best solvent system to screen the phytochemicals and exhibiting the best beneficial activities for the respective phytochemical compounds. The result of the present study showed that the ethanolic leaf extract of *Molohkia* contains highest amount of phenolic compounds exhibited the greatest antioxidant activity. The high scavenging property of *Molohkia* may be due to hydroxyl groups existing in the phenolic compounds.

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دراسة فيتوكيميائية ونشاط مضادات الأكسدة لمستخلص أوراق الملوخية

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قسم الكيمياء الحيوية - كلية الزراعة - جامعة الزقازيق - الزقازيق - مصر

الهدف من هذا البحث هو دراسة نشاط مضادات للأكسدة والتحليل الوصفي والكمي لبعض المركبات الفيتوكيميائية وذلك في مستخلص أوراق الملوخية، كشف التحليل الوصفي لمستخلص اوراق الملوخية عن وجود مركبات مثل (قلويدات، التانينات، الصابونين، الفينول، جليكوسيدات، المنشطات، تربينويدات وقلافونويد) اظهرت النتائج ان مستخلص الايثانول لأوراق الملوخية يحتوي علي اعلي كمية من المركبات الفيتوكيميائية وكذلك أعلي نشاط كمضاد للأكسدة من المستخلصات الاخرى وذلك لوجود العديد من مجاميع الهيدروكسيل الفينولية وكانت نسبة المركبات الفينولية ٩.٣٥ ملجم حمض جاليك /١٠٠جم مستخلص والتانينات ٦.٠١ ملجم حمض جاليك /١٠٠جم مستخلص والقلويدات ٢٨.٥١ ملجم الترويين /١٠٠جم مستخلص والفلافونيدات ١٣.٨٤ ملجم كيرستين/١٠٠جم مستخلص.

المحكمون:

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