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*Full Length Research Paper*

# Correlation of the antioxidant capacity with the phenolic contents of *Hypericum monogynum* and *Hypericum perforatum*

**Niala Raziq[1], Naveed Muhammad[2]*, Kamran Ahmad Chishti[1], Muhammad Saeed[2], Shafiqur Rahman[1] and Haroon Khan[2]**

[1]Department of Pharmacy, Sarhad University of Science and Technology, Peshawar, Pakistan.
[2]Department of Pharmacy, University of Peshawar-25120, Pakistan.

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**This study was carried out to determine the total antioxidant activity and phenolic content of the two plants, that is, *Hypericum monogynum* and *Hypericum perforatum*. The antioxidant activities of both plants were carried out on the crude methanolic and subsequent solvent fractions in two concentrations, that is, 50 and 100 µg/ml. Among the tested samples, 80% methanolic and pure methanolic fractions showed good antioxidant activity in both plants. The antioxidant activity of NR-04 (combination of quercetin and astilbin) showed dose dependent effects and the highest activity was observed at 200 µg/ml. The phenolic contents of *H. monogynum* were quantified in all solvent fractions. The outstanding phenolic contents were found in 80% methanolic (7560 mg/100 g), pure methanolic (6923 mg/100 g), chloroform (5624 mg/100 g) and ethyl acetate fraction (5234 mg/100 g). Similarly *H. perforatum* was screened for phenolic contents, the highest phenolic contents were observed in methanol (7512 mg/100 g), ethyl acetate (7234 mg/100 g), chloroform (6513 mg/100 g) and in methanol 80% (6451 mg/100 g). The correlation between the antioxidant activity and phenolic contents were determined using word Excel. Weak correlation was found between the antioxidant activity and total phenolic contents.**

**Key words:** *Hypericum monogynum, Hypericum monogynum,* antioxidant, phenolic content.

## INTRODUCTION

Biological processes in living organisms are performed by energy produced by oxidation inside the living system. However, the *in-vivo* production of oxygen containing free radicals and other reactive oxygen species, causes cell fatality and tissue injure. Oxidative damage caused by free radicals may be related to aging and diseases, such as atherosclerosis, diabetes, cancer and cirrhosis (Halliwell and Gutteridge, 1984). Approximately all living organisms have antioxidant defense and repair systems to protect them against oxidative damage, these mechanisms are inadequate to avoid the damage entirely (Yang et al., 2002). However, antioxidant supplements, or foods containing antioxidants can protect the human being from oxidative stress and can elevate a large number of diseases. Plants provide us energy and other nutrients but the contributing factors are due to the presence of ascorbic acid, tocopherols and carotenoids. Plants are the rich source of phenolic substances. Phenolic substances are a group of phytonutrients that exercise strong antioxidant properties. They can be classified into simple phenols, phenolic acids, hydroxycinnamic acid derivatives and flavonoids (Ismail et al., 2004).

The genus *Hypericum* L. (family Clusiaceae) comprises about 460 species distributed worldwide in temperate regions. Hypericum species were already known to early communities as helpful therapeutic plants. The well known among them is *Hypericum perforatum* (St. John's wort) and *Hypericum monogynum* the use of which as a medicine was described and recommended throughout the Middle Ages. Several groups of bioactive natural products, *e.g.*, naphthodianthrones, phloroglucinols, phenylpropanoids, flavonol glycosides, biflavones, tannins,

*Corresponding author. E-mail: drnaveedrph@gmail.com.

proanthocyanidins and xanthones have been isolated from the genus. Biological tests of the flavonoids isolated from the genus *Hypericum* demonstrated inhibition of lipoxigenase, (Obložinský, 2006) antidepressant (Butterweck et al., 2004) and scavenging activity, while the flavonoid glycoside exhibited antifungal activity. In Pakistan, twenty species of this genus are known. *H. monogynum* Miller (Syn. *Hypericum chinense* L.) is distributed mainly in S.E. China and Taiwan. It is cultivated in many parts of the world, including the plains of W. Pakistan. This species is used in traditional medicine as an alternative, antidote and astringent, as well as in the treatment of miasmatic diseases.

## MATERIALS AND METHODS

### Plant materials

The aerial parts of *H. monogynum and H. perforatum* were collected at the University of Peshawar campus during the flowering stage in July 2004 and the taxonomic identification was performed by Professor Abdul Rasheed. A voucher specimen (No. 29304-PUP) is deposited in the Herbarium of the Department of Botany, University of Peshawar.

### Extraction and fractionation

#### *Direct crystallization of NR-O4 from the crude extract of H. monogynum*

The air dried and powered aerial parts of the plant (200 g) were percolated with ethyl acetate (3x400 ml) for 24 h at room temperature. The combined extracts were evaporated in vacuo on a rotary evaporator to dryness leaving a brown residue (90 g). This residue was then dissolved in small amount of ethyl acetate and to it added n-hexane (2×200 ml) to defat the ethyl acetate extract. The solution was then poured and the polyphenolic residue was left behind in the container. This residue was again dissolved in small amount of ethyl acetate and chloroform (2×300 ml) was added, after which the solution was shook properly and left for three hours. The solution was filtered and the formation of crystals in the solution after 2 to 3 days was noted. Percent yield ofNR-04 crystals was 0.25.

### Solid phase microwave transformation

NR-04 (0.5 g) dissolved in methanol, was adsorbed on silica gel (2 g). This mixture was dried at 45°C on water bath and transferred to a Pyrex glass. It was then heated it in a microwave oven for 11/2 h. After heating, it was dissolved in ethyl acetate and compared with substrate through thin layer chromatography. Some byproducts also formed with the main product quercetin.

### Antioxidant evaluation of *H. monogynum, H. perforatum* and NR-O4

### Preparation of the extracts

The aerial parts of *H. monogynum* and *H. perforatum*, collected from University of Peshawar and Bara Gali, respectively were left on a bench to dry. 20 g dried sample for each extraction from both plants was chopped in small parts in a blender. Afterwards, it was sequentially extracted with 50 ml of petroleum ether, chloroform, ethyl acetate, acetone, methanol, water: methanol (20:80) and methanol, followed by water three times for 24 h for the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. All the extractions were followed by filtration and evaporation of the filtrate to dryness in rotary evaporator under reduced pressure. NR-04 isolated from the aerial parts of *H. monogymun* was also used for DPPH radical scavenging activity.

### 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging procedure

A 0.1 mM solution of 1, I-Diphenyl-2-picrylhydrazyl (DPPH) in methanol was prepared and I ml of this was added to 3 ml of various quantities of sample and the reference compound. After 30 min, absorbance was measured at 517 nm. Butylated hydroxyl toluene (BHT) was used as a reference material. All tests were performed in triplicate. Percent inhibition was calculated by comparing the absorbance values of control and samples.

### Total phenolic contents

Total phenolic constituents of crude methanolic extract and subsequent solvent fractions were tested for their phenolic content following recommended methods involving Folin-Ciocalteu reagent and gallic acid as standard (Slinkard and Singleton, 1977). Extract solution (0.1 ml) containing 1000 µg extract was taken in a volumetric flask, 46 ml distilled water and 1 ml Folin-Ciocalteu reagent were added and flask was shaken thoroughly. After 3 min, 3 ml of solution 2% $Na_2CO_3$ was added and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm. The same procedure was repeated for gallic acid solutions (0 to 1000 mg, 0.1 $ml^{-1}$) and standard curve was obtained.

## RESULTS AND DISCUSSION

### Direct crystallization of NR-O4 from the crude extract of *H. monogynum*

Direct crystallization from the crude extract of plant material is an easy method for the recovery of compounds as compared to column chromatography and thin layer chromatography. By direct crystallization from the crude extract of ethyl acetate, the yellow colored crystals of NR-04 were formed after 24 h; this process of crystallization was completed within three days. NR-04 was purified by repeated crystallization with methanol and water three times. The percent yield of NR-04 was 0.25 and this NR-04 was a mixture of two compounds, that is, quercetin and astilbin as mentioned in our research paper (Figure 1) (Arfan et al., 2009).

### Solid phase microwave transformation

In the microwave transformation reaction, NR-04 containing mainly quercitrin was converted to quercetin by the removal of glycone part of the quercitrin upon heating.

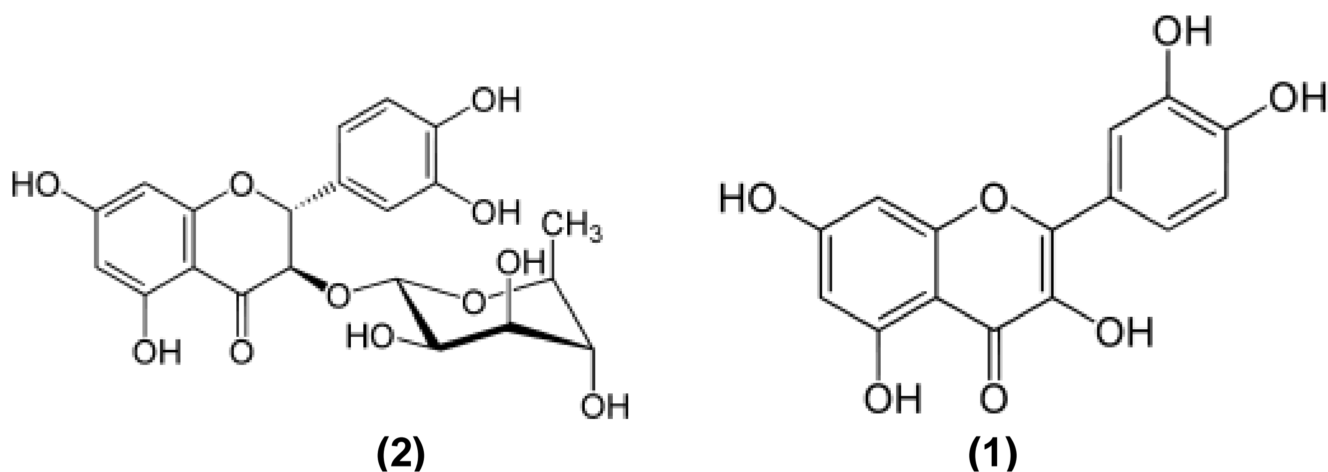


**Figure 1.** Structure of quercetin (1) and astilbin (2).

Some by-products were also formed by this solid phase microwave transformation reaction with the major product formed as quercetin. Quercetin was purified by repeated column chromatography using silica gel as adsorbent. Column was eluted with ethyl acetate and chloroform (0.5:2) solvent system. Quercetin was obtained as yellow powder (mp.184°C). The yield of the compound was found to be 60% and the Rf value is 0.58 (50% EtOAc-n-Hexane). The molecular formula, $C_{15}H_{10}O7$, of quercetin was determined from ESI-MS (m/z 301) together with FAB (+ve and -ve) experiments. By analyzing its UV, IR and comparative $^{1}$H-and $^{13}$C-NMR spectral data with the literature value of the reported compound (Tubitak and Omur, 2005), the structure of quercetin was determined as known compound (3,3',4',5,7-Pentahydroxyflavone).

## Antioxidant profile

In the DPPH radical scavenging method, the ability of an antioxidant to bind the 1,1- diphenyl-2-picrylhydrazyl-radical (a very stable free radical species) is measured, using various concentrations of the selected antioxidants. A compound with high antioxidant potential effectively traps this radical thereby preventing its propagation and the resultant chain reaction. The reference material was butylaled hydroxytoluene (BHT). The antioxidant activities of all the extracts were concentration dependent because all extracts were good antioxidant at higher concentration. The antioxidant activity of *H. monogynum* is shown in Table 1. It is clear from the results that the excellent antioxidant activity was shown by crude methanolic extract followed by methanolic 80% (20% water) with percent inhibition of free radicals 91.00 and 90.56, respectively. The results of antioxidant activity *H. perforatum* are given in Table 2. The significant action was shown against DPPH free radicals by crude pure methanolic extract and 80% methanol in water. The present study suggests that the aerial parts of *H. monogynum* and are potentially rich sources of natural antioxidants.

## Total phenolic contents

Phenolic components are possible antioxidants (Wettasinghe and Shahidi, 1999, Hou et al., 2011). Phenolic compounds can donate hydrogen to free radicals and stop the chain reaction of lipid oxidation at the initial stage. This ability of phenolic compounds to scavenge radicals comes due to the presence of their phenolic hydroxyl groups (Sawa et al., 1999). The correlation between the phenolic content and antioxidant activity has been reported by several authors. Some authors found a strong correlation between the phenolic content and the antioxidant activity, while others found no such relationship (Velioglu et al., 1998). Some researcher reported a strong relationship between total phenolic content and antioxidant activity in selected fruits, vegetables and grain products. No correlation between antioxidant activity and phenolic content was found in some studies (Kähkönen et al., 1999, Ismail et al., 2004) on some plants containing phenolic compounds. The phenolic contents were quantified in all the extracts of both plants. The phenolic content of *H. monogynum* is shown in Figure 2. The highest phenolic contents were observed in 80% methanolic, pure methanolic, chloroform and ethyl acetate fractions having numerical values 7560, 6923, 5624 and 5234 mg/100 g respectively. Among the aforementioned fraction, there are some correlation between antioxidant activity and phenolic contents but this correlation is very weak. The 80% methanolic extracts and pure methanolic extract were excellent antioxidant having highest phenolic contents while the antioxidant activity of chloroform and ethyl acetate was weak although having high phenolic contents. The antioxidant effect of NR 04 is given in Table 3. This compound mixture was found an excellent antioxidant with percent

**Table 1.** Antioxidant and total phenolic contents profile of *H. monogynu.*

| Extract | | Absorbance | Percent inhibition | Total phenolic contents (mg/ 100 g) |
|---|---|---|---|---|
| Petroleum ether (µg/ml) | 50 | 1.67 | 7.22 | 1250 |
| | 100 | 1.54 | 14.44 | |
| Chloroform (µg/ml) | 50 | 1.75 | 2.78 | 5624 |
| | 100 | 1.67 | 7.22 | |
| Ethyl acetate (µg/ml) | 50 | 1.35 | 23.89 | 5234 |
| | 100 | 0.96 | 46.67 | |
| Acetone (µg/ml) | 50 | 1.35 | 25.00 | 2314 |
| | 100 | 0.81 | 55.00 | |
| Methanol (µg/ml) | 50 | 0.40 | 77.78 | 6923 |
| | 100 | 0.15 | 91.67 | |
| Methanol, 80% (µg/ml) | 50 | 0.3 | 83.33 | 7560 |
| | 100 | 0.17 | 90.56 | |
| Water (µg/ml) | 50 | 1.12 | 37.78 | 4421 |
| | 100 | 0.35 | 80.56 | |
| BHT, 50 µg/ml | | 0.29 | 83.89 | |

**Table 2.** Antioxidant and total phenolic contents profile of *H. perforatum*.

| Extract | | Absorbance | Percent inhibition | Total phenolic contents (mg/100 g) |
|---|---|---|---|---|
| Petroleum ether (µg/ml) | 50 | 1.8 | 0.56 | - |
| | 100 | 1.77 | 1.67 | |
| Chloroform (µg/ml) | 50 | 1.78 | 1.11 | 6513 |
| | 100 | 1.74 | 3.33 | |
| Ethyl acetate (µg/ml) | 50 | 1.46 | 18.89 | 7234 |
| | 100 | 1.18 | 33.33 | |
| Acetone (µg/ml) | 50 | 1.39 | 22.76 | 4523 |
| | 100 | 0.94 | 47.78 | |
| Methanol (µg/ml) | 50 | 0.48 | 73.33 | 7512 |
| | 100 | 0.16 | 91.11 | |
| Methanol, 80% (µg/ml) | 50 | 0.54 | 70.00 | 6451 |
| | 100 | 0.18 | 90.00 | |
| Water (µg/ml) | 50 | 1.55 | 19.44 | 2135 |
| | 100 | 1.00 | 50.00 | |
| BHT, 50 µg/ml | | 0.29 | 83.89 | |

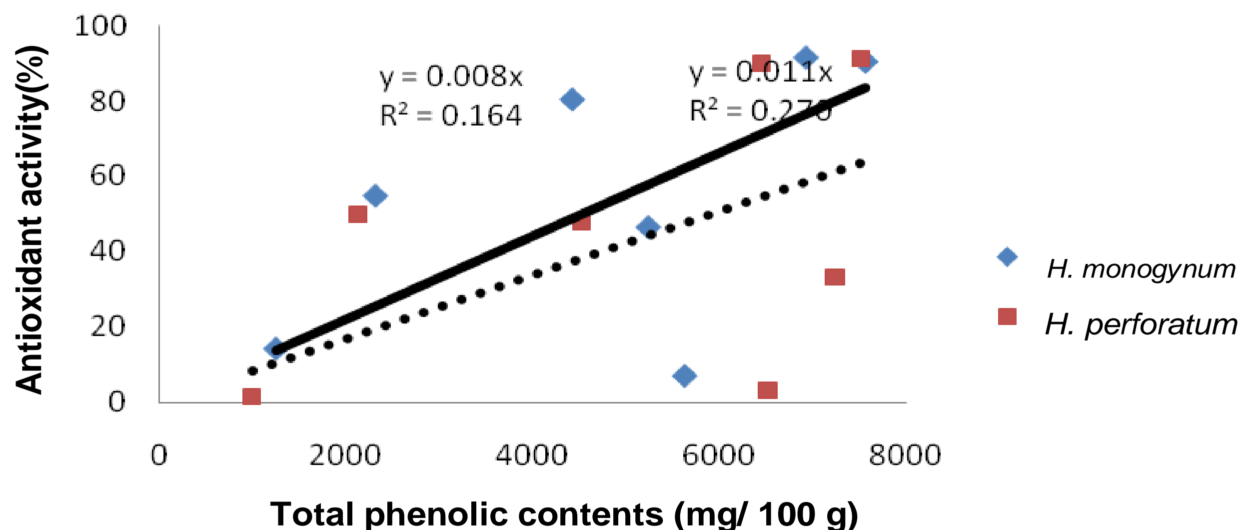


**Figure 2.** Correlation of antioxidant activity and total phenolic content of *H. monogynum and H. perforatum.*

**Table 3.** Antioxidant profile of NR-04.

| Antioxidant profile | | Absorbance | Percent inhibition |
|---|---|---|---|
| NR 04 (µg/ml) | 5 | 1.93 | 12.95 |
| | 12.5 | 1.91 | 37.59 |
| | 25 | 0.44 | 82.73 |
| | 50 | 0.22 | 90.23 |
| | 100 | 0.20 | 91.36 |
| | 200 | 0.18 | 92.27 |
| BHT (µg/ml) | 50 | 0.29 | 83.89 |
| DPPH (mmol) | 0.5 | 2.20 | 00 |

inhibition of 92.27 against free DPPH free radicals.

In conclusion, both plants are good source of phenolic compounds and can be use as antioxidant.

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