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Ethanol And Methanol Extracts of Flowers Of Mirabilis Jalab

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Abstract:
The aim of this study was to investigate and compare Mirabilis jalab L. extracts obtained in Ultrasonic condition with different water/methanol and water/ethanol extraction mixture acidified with 0.1% HCl. The extracts were analyzed for monomeric anthocyanins contents and antioxidant activities. The highest anthocyanins content (3197.8 mg/L) and the best antioxidant activity were obtained for the Mirabilis jalab extract with 100% ethanol. Also, there is a good correlations between antioxidant activity (R^2= 0.9332 and 0.9712) for water/ethanol and methanol series extracts respectively.

Key words*: Mirabilis. anthocyanins, extraction. Antioxidant. Free radical. Scavenging activity.*

1.Introduction

Mirabilis jalapa is an erect herb that can grow up to 1 m tall. Leaves are simple, heart-shaped, 3–12 cm long, opposite, tapering to a pointed end. Flowers are bisexual, red, pink, yellow or white, with perianth distinctly constricted above, and they bloom late in the afternoon. Fruits are black and globose, 5–8 mm in diameter. The use of traditional medicine at the primary health care level is widespread in Yemen; traditional healers use many local plants for the treatment of microbial disease. Mirabilis jalapa, a wild grown plant, is used to cure externally infected wounds. In Latin America, this plant is used in traditional medicine because of the purgative or emetic cathartic properties contained in its roots (Perrot, 1943). In Malagassy, it is used to treat intestinal pains. In South Africa, the roots are used as a purgative drug and it is reputed that the flowers of this plant at night exhale a strong odour which will stupefy or drive away mosquitoes. The roots contain oxymethylanthraquinone (Watt and Breyer-Brandwijk, 1962).Furthermore, it has been reported that Mirabilis jalaba harbor antiviral activity against viruses (Kubo et al, 1990), exhibit antifungal activity (De Bolle et al., 1995; Wang et al., 2001) and showing features as anticarcinogen chemotherapeutic potential. Moreover, in recent years, researches determining the anthocyanin content, relation with chlorophyll and regulation of pigments with genes (Russell., 1997; Kubo et al, 1990; Vivanco et al., 1999; Dela et al., 2003; Halbwirth et al., 2003). Anthocyanins are representative of plant pigments widely distributed in coloured fruits and flowers. Because anthocyanins are widely consumed, finding out additional biological activities related to these compounds would be of great interest (Andreia et al., 2007). Anthocyanins are normally obtained by extraction from plants and the extraction methods currently are employed with the use methanol, ethanol, acetone, water or mixtures as solvents. In fact, the colour stability of anthocyanins depends on a combination of factors, such as the structure and concentration of the anthocyanin, pH, temperature and the presence of complex agents such as phenols and metals (Padma et al., 2010). The most common solvents used for anthocyanins extraction are aqueous mixtures of ethanol, methanol or acetone (Kahkonen et al., 2001). The adverse effects of oxidative stress on human health have become a serious issue (Duduku et al., 2011). Under stress, our bodies produce more reactive oxygen species (ROS) such as; superoxide anion radicals, hydroxyl radicals and hydrogen peroxide than enzymatic antioxidants such as; superoxide dismutase (Riley, 1994)., Glutathione peroxidase, and catalase and non-enzymatic antioxidants such as; ascorbic acids, glutathione, carotenoids, and flavonoids. This imbalance leads to damage of biological structures such as proteins, lipids and DNA and induce a variety of human diseases (Elahi and Matata, 2006; Thrasivoulou et al., 2006; Aruoma, 1998; Lefer and Granger, 2000; Smith et al., 2000; Bhatia et al., 2003; Peuchant et al., 2004). Antioxidants from fruits and vegetables, especially with an intense colouration, are considered an important protection factor against oxidative stress and its deleterious consequences to human health (Vollmannova et al., 2009; Pineli et al., 2011). The antioxidative capacity, which is defined as the capacity to inhibit or delay the oxidation of other molecules, anthocyanins and their aglycones (anthocyanidins) and their free radical scavenging activity have been revealed (Wang et al., 1999; Nakajima et al., 2004; Orak, 2006). The antioxidant activity of berries is directly proportional to the anthocyanins content (Adina et al., 2010). In response to the increased popularity and greater demand for medicinal plants, a number of conservation groups are recommending that wild medicinal plants be brought into cultivation. Various herbs and spices

have been reported to exhibit anthocyanin content in their flowers including Mirabilis jalaba (Padma and Dhara, 2010) and it is reported that, various herbs and species have been reported to exhibit antioxidant activity, Ocimum sanctum, Piper cubeba, Allium sativum, Terminalia bellerica, Camellia sinensis and Zingiber officinale (Norman et al ., 2008). Various analytical methods have been used to evaluate the antioxidant properties of phenolic compounds: the 1, 1-diphenyl- 2-picrylhydrazyl (DPPH) assay proves the capacity of the antioxidants to quench the PPH radical, whereas the ORAC method is based on the loss of fluorescence of the -Phycoerythrin protein or of fluorescein upon oxidation (Cao, et al., 1993; Ninfali, et al., 2005). Reactive oxygen species (ROS), including super oxide anion (O_2^-), hydroxyl radical (OH) and hydrogen peroxide (H_2O_2), exist in living organisms (Riley, 1994). The red colour of the wild Yemeni Mirabilis jalaba is a consequence of its anthocyanin contents that was not well scientifically investigated in extracts in Yemen. Therefore in the present study, Mirabilis jalaba flower plant was collected from the Ibb region in Yemen to study its anthocyanin content and antioxidant properties.

2.Material And Methods

2.1.Material

Fresh petals of the (Mirabilis jalaba) were collected randomly from the Ibb region during June 2013

2.2.Extraction Of Anthocyanins

The anthocyanins were extracted according to the methodology of Lees and Francis (1972). Solvents such as methanol and ethanol were used at concentrations of 100, 75, 50, 25 and 0.0% in water, acidified with 0.1% hydrochloric acid (HCl) (Synth 37%) Twenty five g of fresh petals of Mirabilis jalab were treated with 100 ml of different water/alcohol solutions acidified with 0.1% HCl (Merck, 37%) as extracting material (solid to solvent ratio 1:4 w/v). Each solution was transferred to a 500 ml beaker, covered with parafilm and stored overnight at 4 °C. The mixture was then filtered under vacuum using nº 1 Whatman paper and a Buchner funnel. Filtrate solution was taken and then 200 ml of solvent was added to complete the mixture. This mixture was later filtered and the residue washed with solvent until obtained a total of 500 ml solution. A 5 ml aliquot was removed from each extract, placed in a 50 ml volumetric flask, the volume completed with two buffer solutions: potassium chloride buffer 0.025 M (pH 1.0) and sodium acetate buffer 0.4 M (pH 4.5) and then the absorbance was measured simultaneously at 516 nm and 700 nm after 15 minutes of incubation at room temperature. Absorbance readings were made at room temperature against distilled water as blank was used for measurements.

2.3.Quantitative Determination Of The Anthocyanins

Determination of total monomeric anthocyanins content was quantified using a pH differential method described by Giusti and Wrolstad (2001). The absorbance was measured simultaneously at 516 NM and 700 NM after 15 minutes of incubation at room temperature. Absorbance readings were made at room temperature against distilled water as blank. A Jasco V 530 UV-Vis spectrophotometer was used for measurements. The monomeric anthocyanin pigment concentration was calculated according to the following equation:

Monomeric anthocyanin pigment (mg/L) = (A x MW x DF x 1000) / (εx1) Where A= $(A510–A700)_{pH\ 1.0}$–$(A516–A700)_{pH\ 4.5}$, MW is the molecular weight (449.2) and ε is the molar absorptivity, (26,900) and DF is the dilution factor.

2.4.Determination Of Antioxidant Activity Of The DPPH Method

The 1, 1-diphenyl-2-picrylhydrazine (DPPH) radical scavenging assay was first described by Blois in 1958 and was later modified slightly by numerous researchers. It is one of the most extensively used antioxidant assays of plant samples. DPPH is a stable free radical that reacts with compounds that can donate a hydrogen atom. This method is based on the scavenging of DPPH through the addition of a radical species or an antioxidant that decolourizes the DPPH solution. The antioxidant activity is then measured by the decrease in absorption at 515 NM. In this method, a 0.1mm solution of DPPH in methanol is prepared (4 mg DPPH /100 ml methanol), and then stored at -20 and 2 ml of this solution are added to 0.5 ml of the sample solution in methanol. The mixture was left to stand at room temperature for 30 min in the dark before Absorbance measurement at 517 NM to assess the stability of the coloured reactive action, A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound. The antioxidant activity of the extracts was estimated by the ability to scavenge the DPPH radical. The DPPH concentration in the reaction medium was calculated from the calibration curve (Figure 1) with the following equation determined by linear regression (R^2 = 0.988). $A515=11.\ 368_x$ -0.0437.

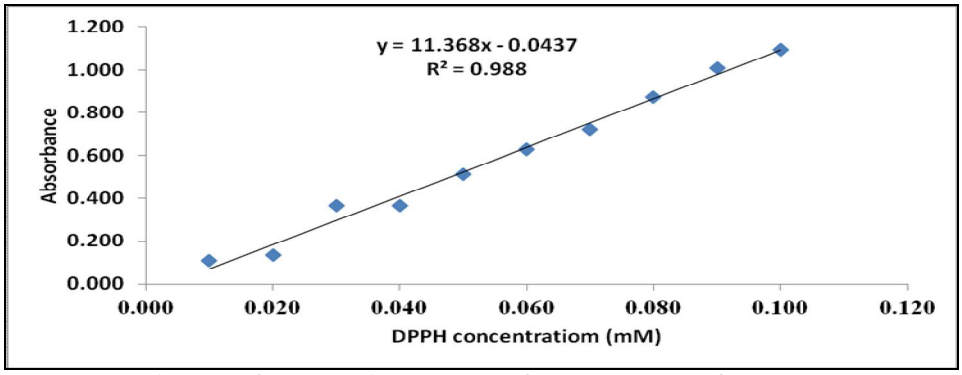

Figure 1: Concentration Response Curve For DPPH At 515 Nm

3.Results And Discussion

The paper describes the extraction method of anthocyanins and antioxidant activity of Mirabilis jalab selected from Yemen. The changes in total anthocyanins content depending on the water/alcohol ratio are shown in Fig. 1. The monomeric anthocyanins content increases with increasing the percentage of methanol in the extraction system. This tendency also observed for water/ethanol extraction, where higher values were obtained for 100 % ethanol extracting system. The amount of monomeric anthocyanins in Mirabilis jalab extracts ranged from 3197.8 mg/L to 55.7 mg/L for water/ethanol extraction, and from 946 mg/L to 278.3 mg/L for water/ethanol extraction.

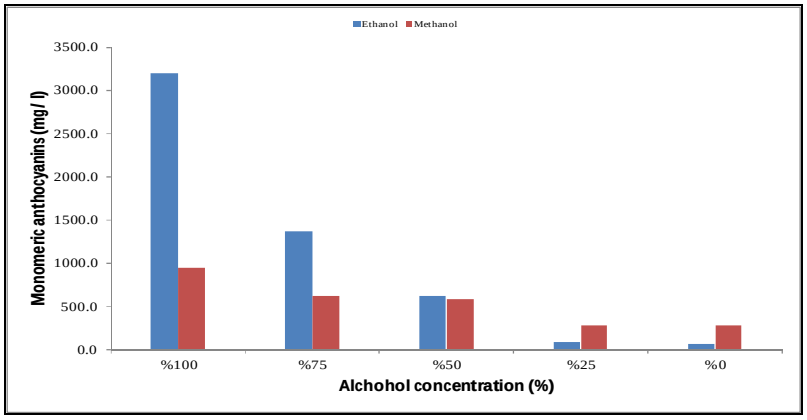

Figure 2: Comparison Of Anthocyanins Content From Extracts Obtained In Water/Methanol And Water/Ethanol Systems

Figure 2 shows the percentage of antioxidant activity after 2 hours of reaction between the extracts and DPPH radical for the two studied cases. The lower this value, the higher is an antiradical efficiency activity of Mirabilis globe extracts increases with increasing the percentage of methanol and ethanol in the extraction system. Comparing antioxidant activities of the Mirabilis jalaba extracts in the two cases, it is observed similar antioxidant activities in the ethanol and methanol series.

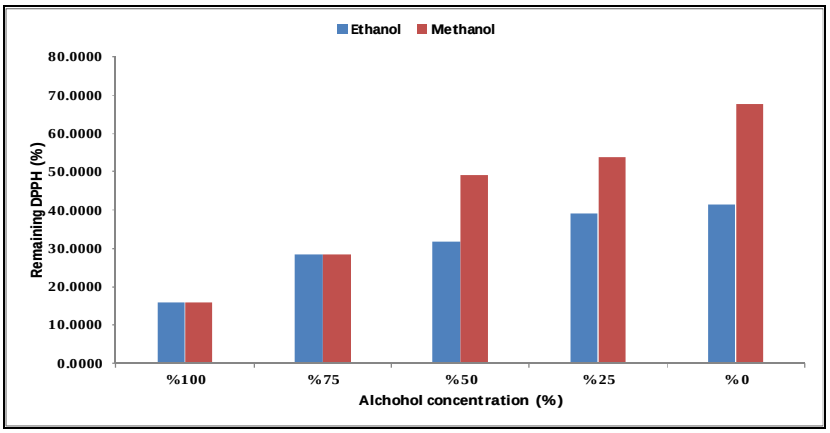

Figure 3:Comparison Of Remaining DPPH Of Extracts Obtained In Water/Methanol And Water/Ethanol Systems

The correlations between antioxidant activity and monomeric anthocyanins content for the two extraction systems are shown in Fig. 3. The following equations determined by linear regression regarding the relationship between antioxidant activity and anthocyanins content was obtained, Eq. (1) for ethanol series and Eq. (2) for methanol series. There is good correlation between antioxidant activity and anthocyanins content for the Mirabilis jalab extracts from methanol and ethanol series.

$y = -0.0077x + 39.478$ ($R^2 = 0.9591$) (1)

$y = -0.0682x + 79.824$ ($R^2 = 0.839$) (2)

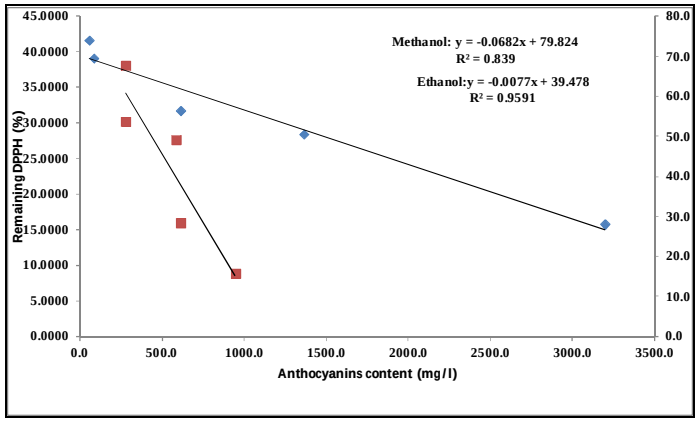

Figure 4: Correlation Between Anthocyanin Contents And Anti Oxidant Activity

Also, notice a good correlation between anti oxidant activity and extraction systems content for ethanol and methanol series extracts. The values of the determination coefficients are acceptable, indicating a high correlation by linear regressions between antioxidant activities and extraction systems Eq. (3) for ethanol series and Eq. (4) for methanol series

$y = -24.938x + 43.818$ $R^2 = 0.9332$ (3)

$y = -51.636x + 68.801$ ($R^2 = 0.9712$) (4)

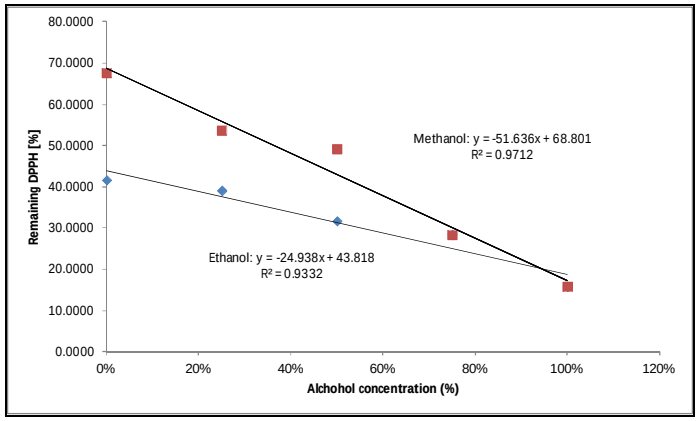

Figure 5: Correlation Between Alcohol Concentrations And Anti Oxidant Activity

The anti oxidant activity by the studied extracts show similar pattern curves of anti oxidant activity versus time. The reaction occurs rapidly in the first minutes and then slowed. The lower step can be due to the antioxidant properties of the slow reacting components originally present in the sample and/or due to the reaction products formed during rapid phase (Tsimogiannis and Oreopoulou, 2006). The percentage of free radical scavenging activity against reaction time is exemplified in Fig. 5and 6 for the extracts obtained in water/methanol system and water/ethanol system respectively.

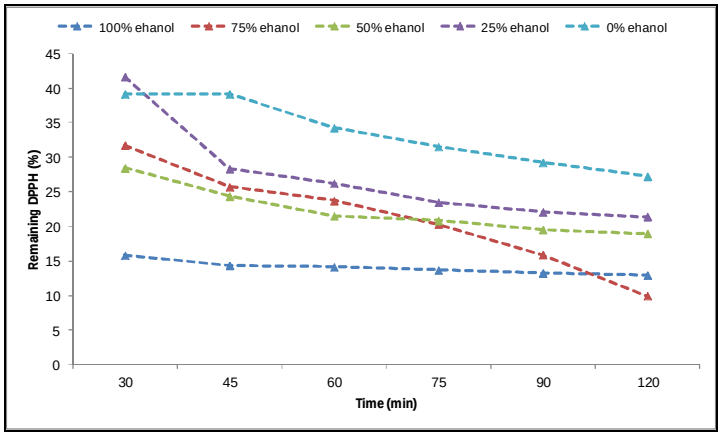

Figure 6: DPPH Scavenging Kinetic Curves For Mirabilis Jalaba Extracts Obtained In Water/Ethanol System

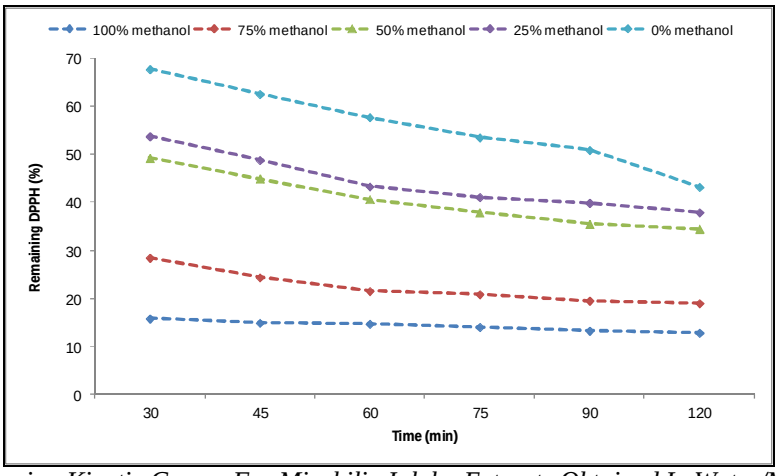

Figure 7: Scavenging Kinetic Curves For Mirabilis Jalaba Extracts Obtained In Water/Methanol System

4.Conclusion

The performed studies indicate that Mirabilis jalab extracts are a rich source of anthocyanins and possess a significant anti oxidant activity. The best results regarding monomeric anthocyanins content and antioxidant activity were obtained at extraction with 100% methanol and ethanol. However, for food industry, the extractions with ethanolic solution are more convenient. The correlations between anthocyanins content, and antioxidant activity depend on the extraction solvent, the best determination coefficients was found for Mirabilis jalab extracts obtained in water/ethanol systems Fig 2.

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