

INFLUENCE OF pH AND TEMPERATURE ON TOTAL PHENOL CONTENT OF *OCIMUM SANCTUM* LEAVES

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ABSTRACT

Ocimum is an important aromatic herb of the Lamiaceae family. It is reported to associate with diverse phenolic compounds with strong antioxidant activity. The antioxidant effect is mainly due to phenolic components such as flavonoids, phenolic acids and phenolic diterpenes etc., The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. Many studies demonstrated a possible relationship between phenolic content and antioxidant activity. In the present study, total phenolic content of *Ocimum sanctum* was estimated at varying pH and temperature conditions to know the stability of phenolics. The methanolic leaf extract of *O.Sanctum* showed a maximum phenolic content of 135mgGAE/gm extract at pH7.2 as against least phenolic content of 75mgGAE/gm extract at pH7.6. The phenolic content was also measured at various temperatures ranging from 10-52^oC and a maximum value of phenolic content was observed at 32^oC. With the above results it was concluded that pH and temperature have a considerable influence over the antioxidant activity of methanolic leaf extract of *O.Sanctum* plant.

Keywords: *Ocimum sanctum*, Total phenolic content, Flavonoids, Antioxidant activity.

INTRODUCTION

Among the various medicinal and culinary herbs, some endemic species are of particular interest because they may be used for the production of raw materials or preparations containing phytochemicals with significant antioxidant capacities and health benefits [1]. The preservative effect of many plant species and herbs suggests the presence of oxidative and antimicrobial constituents in their tissues [2-5]. Many medicinal plants contain large amounts of antioxidants other than vitamin C, vitamin E and carotenoids [6]. Most of the antioxidative potential of herbs and spices is due to the redox properties of their phenolic compounds, which permit them to act as reducing agents, hydrogen donors and singlet oxygen quenchers [7]. Plant phenolic compounds are mostly secondary metabolites possessing high antioxidant activity and are wide spread in the species of Lamiaceae [8]. Many works have been carried out in finding natural antioxidants for use in foods or medicinal preparations rather than synthetic antioxidants which may be deleterious to health. Many phenolic compounds have been reported to possess

potent antioxidant activity and to have anticarcinogenic, antibacterial, antiviral and anti-inflammatory activities

This activity is mainly due to their redox properties which can play an important role in absorbing and neutralizing free radicals [9-12] quenching singlet and triplet oxygen and decomposing radicals [13]. Plant extracts and plant derived antioxidants can elicit a number of in-vivo effects such as promotion of increased synthesis of endogenous antioxidant defense or themselves acting directly as antioxidants.

Many studies indicated that *O.Sanctum* has antistress, antioxidant, hepatoprotective, immunomodulating, anti-inflammatory, antibacterial, antiviral antifungal, antidiabetic, antimalarial and hypolipidemic properties [14]. All the above medicinal properties of this plant were expected to be associated with the diversified secondary metabolites such as flavonoids and phenolic compounds. Fresh leaves or leaf extract is consumed orally to get the beneficial medicinal effects of

the plant. However when consumed orally, bioactive compounds are subjected to various pH conditions of the human digestive tract and are also subjected to various temperature conditions in different individuals of different age groups. Thus, there exists a need to investigate the effects of various pH and temperature conditions on the antioxidant status of plant extracts.

Extraction of active phenolics from plant tissues depends on various extraction procedures [15]. Most phenolics present in plant tissues are soluble in polar solvents such as water, methanol, ethanol etc. [16, 17]. Phenol compounds show good antioxidant ability [18] but they are relatively unstable [19]. The stability of phenol compounds is dependent on various factors such as pH and temperature [20]. Thus an investigation of the stability of phenolics from *O.Sanctum* requires evaluation under various temperatures and solvent pH values. In this context, we have attempted to know the influence of pH and temperature on the total phenolic content and antioxidant activity of methanolic leaf extract of *O.Sanctum*.

MATERIALS and METHODS

Preparation of plant extract:

Fresh leaves of *O.Sanctum* were collected from our institute's garden. Leaves were washed in running water and are dried under shade for 1 week and later ground to a fine powder. About 50 gm of this powder was soaked in 300 ml of 80% aqueous methanol and kept in a shaking incubator at room temperature for 48 hours. Later, the methanolic extract was concentrated under reduced pressure in a rotary evaporator. The extract was stored in a glass container at 4 °C for further use.

Total phenols estimation

The total phenol content (TPC) of the methanolic extract (0.5 gm dry extract in 10 ml of DMSO/water solution, 1:1) of *O.Sanctum* leaves was estimated by Folin-ciocalteu method [21]. To 1 ml of dilute plant extract (0.5 mg/ml) with a pH of 7.2, 5 ml of dilute FC reagent and 4 ml of 7.5% sodium carbonate are added and mixed thoroughly. The mixture was allowed to stand for 90 min. at room temperature for colour development. Absorbance was measured using UV-visible spectrophotometer at 765 nm. A standard curve was prepared by using 250 µg/ml of gallic acid in ethanol as standard. The concentration of phenolic compounds was expressed in terms of gallic acid equivalents (mgGAE/gm dry extract).

Effect of various pH conditions on total phenol content

The methanolic leaf extract of *O.Sanctum* at an initial pH of 7.2 was dialysed with buffer solutions of acidic and alkaline pH values- 2.0, 6.0, 6.5, 7.6 and 8.2 so as to attain the respective pH values under stabilized conditions. Later the total phenolic content was estimated in all the respective solutions of different pH values by FC

method as described above. The concentration of phenolic content of all the solutions was expressed in terms of GAE/gm extract by using standard curve.

Effect of various temperatures on total phenol content

The dilute methanolic leaf extract of *O.Sanctum* leaves was then used to examine the effect of temperature conditions on the total phenol content. The methanolic leaf extract was incubated at 10, 22, 32, 44 and 52 °C and then tested for total phenolic content by FC method. The absorbance of all the solutions was measured at 765 nm and the values expressed in terms of GAE (mg/gm extract) using the standard curve.

RESULTS AND DISCUSSION

In the present study, the total phenolic content of the *Ocimum* leaf extract was studied with respect to different pH and temperature conditions. Figure 1 shows the results for total phenolic content of the leaf extract at various acidic and alkaline pH conditions.

As per the above results, it was verified that the total phenolic content showed a considerably high value of 135 mgGAE/gm at pH 7.2 as against a lowest value of 75 GAE mg/gm at pH 7.6. These results indicate that pH 7.2 can be considered as the best pH to prepare the *Ocimum* leaf extract for further systematic study.

The methanolic extract of *O.Sanctum* leaves was tested for the total phenol content at various temperatures ranging from 10-52 °C and analysed for antioxidant activity. Results (figure 2) indicate that the extract showed a least value of 130 mg GAE/gm extract at 10 °C, increasing with increase in temperature upto 32 °C, after which there was a slight decline in the total phenol content with a value of 145 mgGAE/gm at 52 °C.

DISCUSSION

Most phenolic acids have antioxidant capacity and the radical scavenging ability of phenolic acids depends on the number and position of hydroxyl groups and methoxy substituents in the molecules [22]. Variations in pH and temperature conditions may alter the chemical structure of the phenolic compounds and thus a change in their antioxidant activity.

The antioxidant properties are dependent on the pH of the medium since changes in pKa values correspond to the change of ionization of hydroxyl groups or other functional groups of the phenolic compounds [23]. Earlier researches reported that the oxidation rate of antioxidant compounds was influenced by the surrounding pH [24]. The TPC of our leaf extract showed a considerably high value at pH 6.06 which indicates that a better antioxidant capacity can also be obtained at that pH. An increase of pH value by 0.5 units decreased the value by 5 mgGAE/gm. Interestingly, the leaf extract showed a good total phenol content even at a highly acidic pH of 2.0 (120 mg GAE) which suggests the probable activation of phenolics at that

pH. However, when the extract was tested for TPC at an alkaline pH of 7.2, a maximum phenolic content of 135mgGAE/gm was obtained. There was a significant decrease in TPC with an increase of pH to 7.6 where only 75 mgGAE/gm was obtained.

Temperature is one of the most important factors affecting antioxidant activity [25]. Generally heating causes an acceleration of the initiation reactions and hence a decrease in the activity of the present or added antioxidants [26]. However variations in temperature may change the mechanism of action of some antioxidants [27] or effect them in another way. Results from fig.2 showed that the TPC of the leaf extract recorded a least phenolic

content at 10⁰C indicating a poor antioxidant activity at that temperature. As temperature was increased, there was a simultaneous rise in the TPC upto 32⁰C, where a maximum value of 160mgGAE was obtained. Thereafter, the results showed a slight decline in TPC with an increase of temperature upto 52⁰C. The above results indicated that the best suitable temperature for obtaining the maximum antioxidant power from the leaf extract was 32⁰C where the phenolic content was found to be 160mg GAE/gm extract. These results proved that thermal processing can induce the formation of novel compounds with antioxidant properties or improve the antioxidant activity of naturally occurring antioxidants [28].

Fig 1. Effect of pH on TPC of *O.Sanctum*

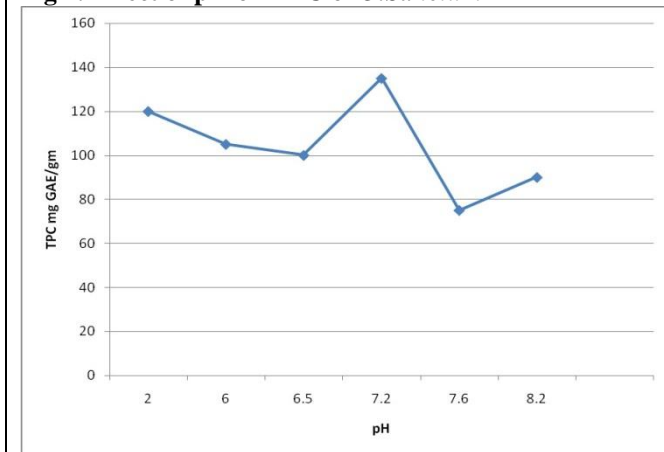
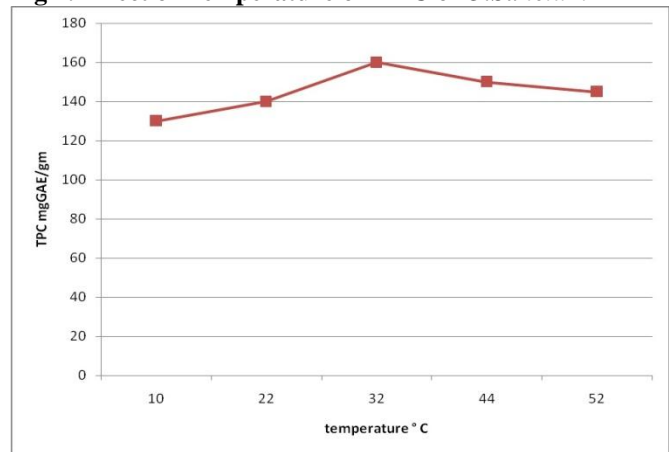


Fig 2. Effect of Temperature on TPC of *O.Sanctum*



CONCLUSION

These studies on *O.s* methanolic leaf extract with respect to pH and temperature variations represented the existence of relationship between the relative increase in antioxidant activity with increasing pH and temperature. Application of pH 7.2 exhibited the most efficient antioxidant activity with highest total phenol concentration. Furthermore the temperatures ranging from 30-50⁰C and pH values from 7-8 exhibited a relatively high antioxidant activity. To conclude, the present study may help in further investigation of the antioxidant power of the

O.Sanctum extract at an extended range of pH and temperature conditions for its utilization in food and biological systems.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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