



## TEMPERATURE AND PH STABILITY OF POLYPHENOLS FROM *MORINDA CITRIFOLIA* LEAF EXTRACT

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### ABSTRACT

*Morinda citrifolia*-MC (*Noni tree*) is a medicinal plant consumed as extracts from fruits, leaves, flowers, bark and roots. In present study, polyphenol content of *Morinda citrifolia* leaves in fresh, dehydrated and 100% methanol extract was analyzed. In the MC-leaves methanol extract, polyphenols stability was also analyzed at different temperature (50°C, 75°C & 100°C) and pH (4, 7 & 9). The polyphenol content was in the order of 0.21% (fresh leaves) < 3.34% (dehydrated leaves) < 3.9% (Methanol extract of leaves). Temperature treatment resulted in 100% stability of polyphenols at 50°C and at 75°C & 100°C around 90% retention of polyphenols was observed. In case of pH treatment, polyphenol content reduced to some extent at all 3 different pH for 4 days. The polyphenol content at 4 pH reduced from 2.33mg/ml to 1.59mg/ml, at 7 pH 2.33mg/ml to 1.88mg/ml and at 9 pH 2.33mg/ml to 2.16mg/ml. Among all, at 9 pH polyphenols were more stable. The results of the study indicate that *Morinda citrifolia* is a good source of polyphenols and retention of polyphenols were observed at various temperatures and pH. Hence the leaf can be used as nutraceutical source in preparing functional food formulations subjected to various processing conditions.

**KEYWORDS:** *Morinda citrifolia*, Polyphenols, nutraceuticals, pH and temperature.

### INTRODUCTION

Plant -based, traditional medicine systems are playing essential role in health care, with about 80% of the world's inhabitants depending mainly on traditional medicines for their primary health care.<sup>[1-4]</sup> World Health Organization (1998)<sup>[5]</sup> defines 'a medicinal plant' as "a plant which has been used for medical purposes at one time or another, and which, although not necessarily a product or available for marketing, is the original material of herbal medicines". The medicinal properties of plants is based on the effects of the phytochemicals such as polyphenols, glutathione, anthocyanins, beta-carotenoids, catechins, flavonoids, indoles, isothiocyanates, isoflavones, lignans, lycopene, phenolic acids, vitamin C, and vitamin E. Among them, polyphenol group of phytochemical substances of particular have gained increasing importance over the past years. In recent years, special attention has been focused on the isolation of phenolics from different raw materials (medicinal plants, fruits, vegetables, industrial by- products, and beverages) and on exploration of their potential benefits for human health.<sup>[6]</sup>

During processing and storage, polyphenols are widely seen as very unstable and highly susceptible to

degradation and/or reaction with some factors (e.g., oxygen and metal ions) leading to structural changes. The stability of polyphenols under different conditions is a very important aspect which has to be taken into account to ensure that phenolic compounds have the desired properties and maintain their activity and structure during the different stages of processing, which can involve high temperatures, light, oxygen, solvents, the presence of enzymes, proteins, metallic ions, or association with other food constituents. These factors affect the biological activity of polyphenols.<sup>[7-9]</sup> Studies have reported the stability of polyphenols to various processing and storage conditions, where freeze drying found to be best method for the stability of phenolic in dried pomegranate aril production.<sup>[10]</sup> Another study found the pericarp of the litchi cultivar loss is red color because of the degradation or loss of stability of anthocyanins caused by high pH and temperature.<sup>[11]</sup>

*Morinda citrifolia* (*Noni tree* is the common name) is a medicinal plant originates from Southeast Asia such as Indonesia and Australia and it is normally grown for roots, leaves and fruits. *M. citrifolia* is consumed as extracts form from fruits although leaves, flowers, bark and roots are also used. These parts contain polyphenols

with medicinal properties that are; leaves (flavonol, iridoid, citrifolinoside) which are anti-cancer. Fruits and fruit juice (glucuronic acid, galactose, arabinose and rhamose) which are good for immune stimulatory and modulatory, anti-bacterial, tumour and cancer and good for wound healing, premature of graying hair and useful for health. Roots contain Anthraquinones (active Damnacanthal) good for antiseptic and anti-bacterial effect.<sup>[12]</sup> Many studies have reported on the nutritional and health values.<sup>[13]</sup> However, there are few limited studies on polyphenols stability to temperature and pH in *M. citrifolia*. Due to the limited information regarding the effect of temperature and pH on polyphenols chemical substances from *M. citrifolia* extract, present study was planned to determine the effect of temperature and pH on stability of total polyphenol content.

## MATERIALS AND METHODS

### Plant Material

*Morinda citrifolia* -MC leaves was collected and the sample was identified by Dr. Janardhan, Department of Studies in Botany, University of Mysore. The collected leaves were washed, dried in the oven overnight at 50° C, powdered, passed through 60 mesh, and stored at 4° C till further use.

**Preparation of 100% methanol extract-** A 15 g MC leaf powder was extracted with 50mL methanol for 6 h in a mechanical shaker. The extracts were filtered and filtrates were evaporated at 40° C under reduced pressure to dryness in a rotary evaporator (Superfit, India). The

residue of extract was stored in air tight container at 4° C until used.

**Estimation of polyphenols:** In fresh leaves, dry powder and 100% methanol extract of *Morinda citrifolia* Polyphenols content was estimated by Folin ciocalteu method by using Gallic acid as standard.<sup>[14]</sup>

**Heat and pH Stability** <sup>[15]</sup>: 10 mg of the methanol extract was dissolved in 10 mL of methanol. The extracts were subjected to different temperature i.e., 50 °C, 75 °C and 100 °C for 15 minutes and 30 minutes. The 50 °C and 75 °C at both 15 minutes and 30 minutes of incubation were done in the hot-air oven while the 100 °C at both 15 and 30 minutes of incubation was done in the water-bath. The polyphenols content was measured in the extract after subjecting to different temperature and time. For pH stability, the methanol extract was incubated for 4 days at pH 4, 7 and 9 buffers and the polyphenol content was determined every 24hrs for 4days.

**Statistical Analysis:** The results are presented as the mean ± standard deviation for the three replicates. n=3.

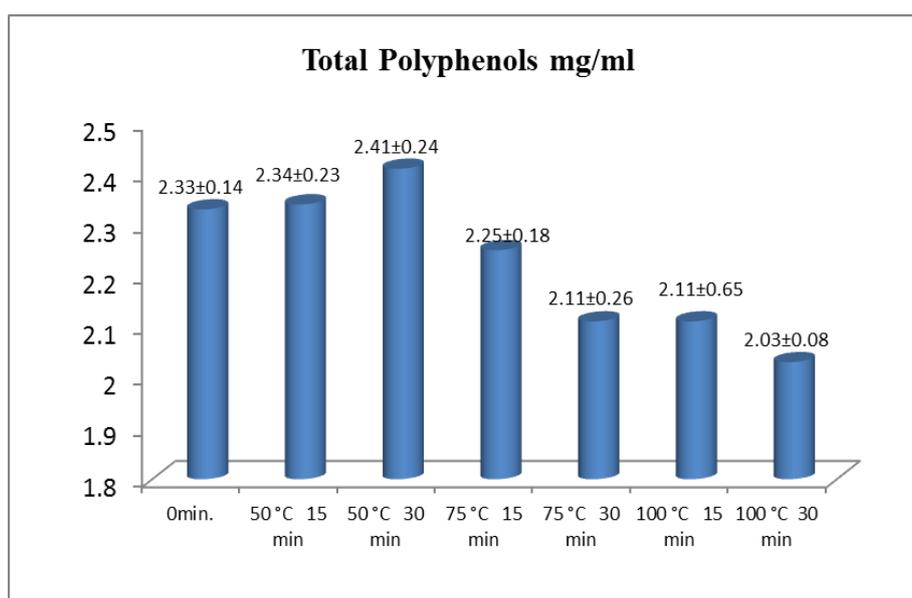
## RESULTS AND DISCUSSION

### Polyphenols content of *Morinda citrifolia*

In fresh and dry leaves powder and 100% methanol extract polyphenol content in the samples ranged between 0.21g to 4.0 g. The total polyphenols content is given in Table 1.

**Table 1: The polyphenols content of *Morinda citrifolia* leaves different forms.**

<i>Morinda Citrifolia</i> Sample	Polyphenols in mg/100 g	g%
Fresh Leaves	209.0	0.21
Dry Powder Sample	3337.5	3.34
100 % Methanol Extract	3958.34	4.00



**Figure 1 Stability of *Morinda citrifolia* leaves methanol extract polyphenol at different temperature.**

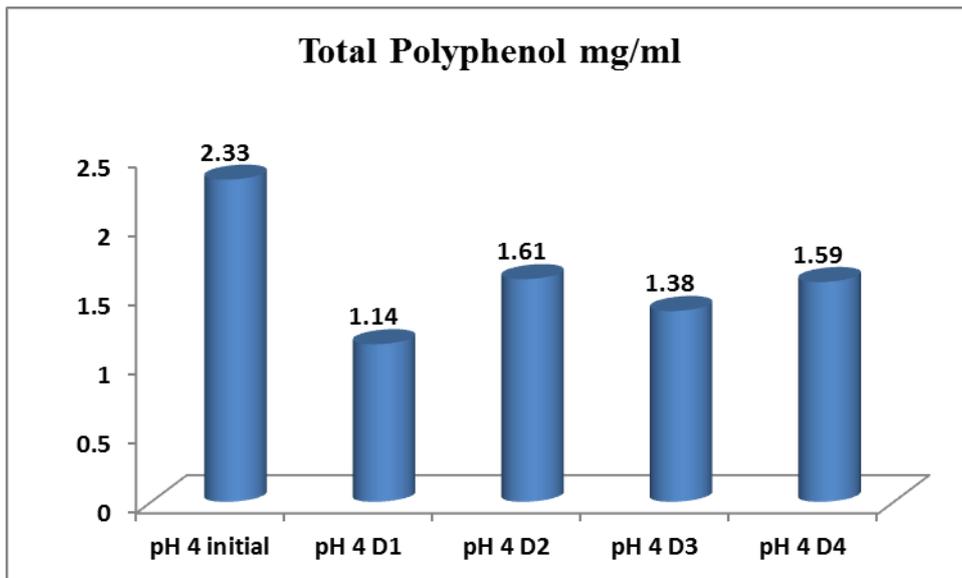


Figure 2: Stability of *Morinda citrifolia* leaves methanol extract polyphenol at pH 4.

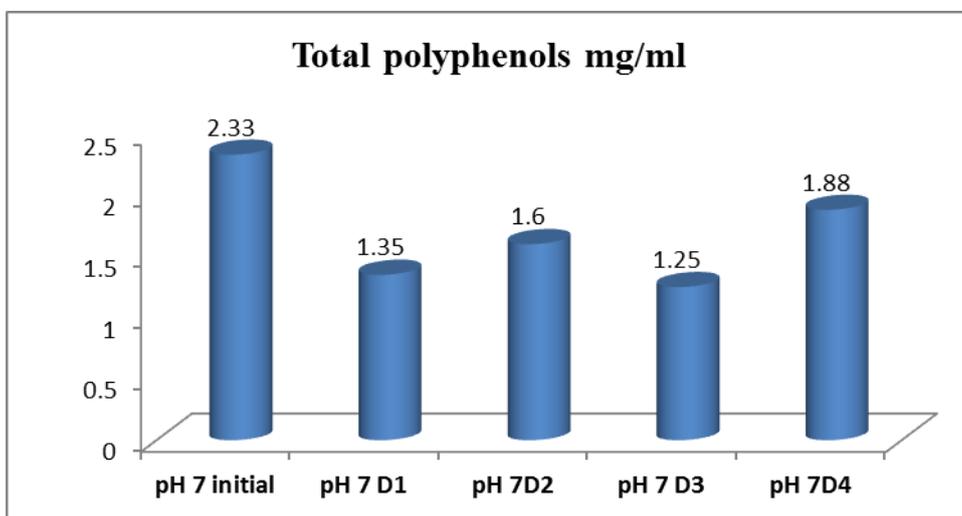


Figure 3: Stability of *Morinda citrifolia* leaves methanol extract polyphenol at pH 7.

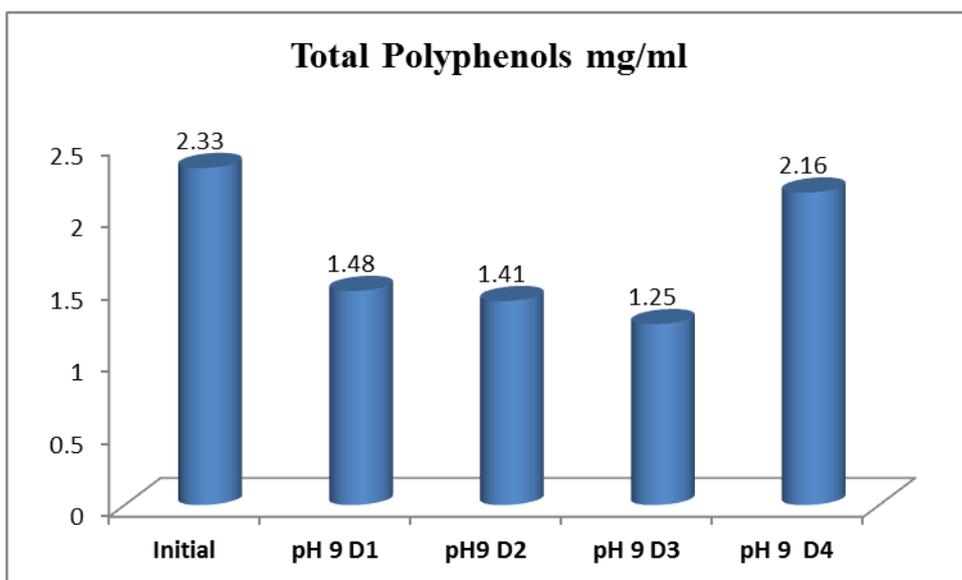


Figure 4: Stability of *Morinda citrifolia* leaves methanol extract polyphenol at pH 9.

### Heat stability

The table of the total polyphenols content of the MC leaf methanol extract after temperature treatment at 0<sup>th</sup> day to 4<sup>th</sup> day is given in figure 1. From the figure it can be observed that 50° C did not affect the polyphenol content for 15 minute and at the end of 30min. an increase in polyphenol content was observed from 2.33 to 2.41mg/ml which may be due to structural rearrangement in polyphenols by application of moderate heat. However the increase was not significant. At 75 °C and 100 °C treatment a decline in polyphenol content was observed at both the time intervals. The polyphenol content reduced was less at 75 °C -2.33mg/10ml to 2.11mg/10ml (90% retention) and more at 100 °C 2.11mg/10ml to 2.03mg/10ml (87% retention).

### Stability at 4, 7 and 9 pH

The Polyphenol stability to different pH for 4 days measured at 24hr intervals is given in figure no. 2, 3 and 4.

#### Stability at pH 4

The total polyphenols content treated at 4 pH for 4 days (D1 to D4) is given in figure 2. From the figure it can be observed that the changes in polyphenol content was not stable. At the end of D1 the polyphenols reduced significantly from 2.33mg/ml to 1.14mg/ml. Again the value increased by the end of D2 and decreased by the end of D3 and finally at the end of D4 the polyphenol content was 1.59mg/ml.

#### Stability at pH-7

The total polyphenol content treated at 7 pH for 4 days (D1 to D4) is given in figure 3. At pH-7, the total polyphenol content decreased by the end of D1 (2.33mg/ml to 1.35mg/ml). By the end of D2 the polyphenol content is increased and reduced by the end of D3 and by the end of D4 the polyphenol content was 1.88mg/ml.

#### Stability at pH-9

The total polyphenols content treated at 9 pH for 4 days (D1 to D4) is given in figure 4. The polyphenol content reduced gradually from 0<sup>th</sup> day (2.33mg/ml) to D3 (1.25mg/ml). At the end of D4 the polyphenol content increased to 2.16mg/ml.

The overall treatment of pH 4, 7 and 9 has resulted in a decrease in polyphenol content in MC leaf methanol extract. Among all pH the MC leaf extract was more stable at pH 9. The percentage of retention of polyphenol content by the end of 4<sup>th</sup> day was at 4 pH- 68%, 7 pH- 81% and at pH 9- 92%. The changes in polyphenol content during the incubation period of 4 days may be due to changes in the structure of phenolic acids at different pH.

The stability of polyphenols under different conditions is a very important aspect which has to be taken into account to ensure that phenolic compounds have the

desired properties and maintain their activity and structure during the different stages of processing, which can involve high temperatures, light, oxygen, solvents, the presence of enzymes, proteins, metallic ions, or association with other food constituents<sup>[9]</sup>. The present study demonstrates that polyphenols are not completely stable at different temperature and pH. However, 100% retention of polyphenols was observed at 50° C and at 75° C and 100° C around 90% retention was noticed. In a study pH and temperature treatment resulted in an irreversible spectral transformation of polyphenols. According to the study, the stability of the phenolic compounds strongly depended not only on the pH of the buffers and storage time but also on the structure of the phenolic compound<sup>[16]</sup>. Another study reported that; the stability of polyphenols depends on many factors, for example, the stability of anthocyanins depends on factors such as temperature, pH, light, oxygen and Ascorbic acid<sup>[10]</sup>. In present study also polyphenols responded variedly at different pH and temperature. According to Crowe<sup>[17]</sup> Phenolic compounds are relatively unstable in comparison to other secondary plant bioactive or plant metabolites such that they are easily degraded during food processing, diet formulation and digestion. Another study, addition of on *Morinda citrifolia* crude extract to canned product resulted in a low reduction of total polyphenol content<sup>[13]</sup>. Our earlier studies have reported on the effect of temperature and pH on the antioxidant activity of various medicinal plants<sup>[15,18]</sup>. There is a need to study the correlation between the polyphenol content and antioxidant activity to confirm the stability of polyphenols to various temperature and pH.

### CONCLUSION

The study reveals that the MC is a good source of polyphenols. The polyphenols were found to be stable to different temperature and pH conditions, which indicates, the plant or the extract is suitable for incorporation or using as supplement in food formulations or therapeutic food undergoing different processing condition. The results indicate, MC can be a nutraceutical with antioxidant property.

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