CHAPTER 3

To conduct a comparative study of the nutritional and phytochemical properties of edible flowers

3.1. Introduction

Edible flowers are a natural source of minerals, vitamin, phytochemicals and various bioactive compounds. They also provide aesthetic appearance, a variety of fragrance, and flavor to dishes. There are more than 97 families encompassing 100 genera and 180 species of edible flowers available worldwide (Lu et al., 2016), where their sources generally includes ornamental plants, fruit and vegetable trees, medicinal and aromatic plants (Acikgoz, 2017) etc. Different cultures such as Indian, Chinese, Romans, Middle Eastern, North American and European etc. use edible flowers in cooking (Fernandes et al., 2017). Based on species to species, some flowers can be eaten whole and some flowers can be eaten for their specific parts only. Edible flowers have been traditionally consumed since time immemorial in various ways such as fresh or processed (in the form of curry, steamed, boiled or deep fried etc.) and in modern days the demand for edible flowers is increasing day by day. Usually, they are sold fresh and also in dried or crystallized form.

Edible flowers comprise both nutritional and medicinal properties. Focusing on nutritional properties, they contain protein, carbohydrates, saturated and unsaturated lipids, sugars, organic acids, antioxidants, phytochemicals (carotenoids, flavonols, alkaloids and terpenoids etc.), vitamins, minerals etc. (Fernandes et al., 2017) making them a rich source of nutrients. Edible flowers have been reported to possess various medicinal properties such as antimutagenic effect, antitumor and anti-inflammatory properties (Benvenuti et al., 2016), antibacterial, antifungal and antiviral effects etc. (Rachkeeree et al., 2018). Additionally, there is a traditional believe that edible flowers cure diseases. Some common edible flowers of Assam, India are *Carica papaya*, *Gmelina arboria*, *Phlogacanthus thyrsiformis*, *Dendrocnide sinuate*, *Justicia adhatoda*, *Oroxylum indicum*, *Nyctanthus arbortristis*, *Phlogacanthus curviflorus*, *Lasia spinosa*, *Alocasia acuminate*, *Cucurbita moschata*, *Tabernaemontana divaricata*, *Canna indica*, *Bauhinia* spp., *Sesbania grandiflora*, *Moringa oleifera* etc.

Flowers of Night jasmine *(Nyctanthus arbortristis),* Drumstick *(Moringa oleifera),* Pumpkin *(Cucurbita* spp.*)* and Nongmangkha *(Phlogacanthus thyrsiflorus)* are commonly consumed in Assam, India. Pumpkin belongs to *Cucurbitaceae* family (genus *cucurbita*) and bears a bright color (between yellow and orange color) and it starts to bloom from February to March. This flower was traditionally believed to be used for cold, male infertility, eye problems, bone formation and improving immunity (Ghosh et al., 2021). Specially, Pumpkin flower contains sodium, potassium and calcium and is a natural source of oleic acid, myristic acid and stearic acid. Santos et al. (2022) mentioned in their study that addition of Pumpkin (*Cucurbita maxima*) flower powder in chicken patties could improve the antioxidant and sensorial properties.

The yellowish white flowers of Drumstick have fragrance and belong to family *Moringaceae* under the genus *Moringa* and generally bloom from January to March. Also, these flowers contain protein, amino acid (Sánchez-Machado et al., 2010), potassium, calcium, antioxidant (α, γtocopherol) (Liu et al., 2018). It was reported that they act as antioxidative agent, exhibits anti inflammatory activity, have diuretic action, offers hepatoprotective effect; prevent asthma, muscle and spleen disease; and effect on and tumors (SiqueiraPatriota et al., 2020) etc. Studies found that extracts of Drumstick flower helps to reduce the oxidation of lipid in cooked chicken nuggets (Madane et al., 2019).

The small, delightful fragrance bearing flower Night jasmine belongs to the family Oleaceae, native to Southern Asia which blooms at night during September to January. It has a bitter, astringent taste (Sasmal et al., 2007) and is reported to have flavonoids, anthocyanins and an essential oil (Jain et al., 2016), potassium, calcium, magnesium, sodium, iron and zinc etc. (Haque et al., 2019). Traditionally this plant is regarded as a medicinal plant and the flowers have been reported to deliver antibilious, antifilarial, anti-inflammatory, antioxidant activity, diuretic, dyspepsia, ophthalmic, sedative effects, cytotoxic activity (Khatune et al., 2001), antibacterial and hepatoprotective (Pal et al., 2019).

Red brick colored Nongmangkha flowers belong to acanthaceae family and an important medicinal plant that blooms from February to April. These flowers were reported to deliver beneficial health effects on hyperlipidemia (Chakravarty et al., 2014), and were showed antioxidant and radical scavenging activities (Nongthombam et al., 2018) and possessed hypoglycemic and hypolipidemic properties (Ahmed et al., 2016). Nongmangkha flowers were believed to cure pox; prevent skin disease like sore, scabies, haveanti-allergic effect, treat wounds and tumours, act as a blood purifier (Koushik et al., 2020), kidney stones and liver disorders (Das et al., 2017). The flowers were reported to contain steroids, terpenoids, flavonoids and phenol etc. (Nongthombam et al., 2018).

Though, edible flowers are abundantly available all over the world but their consumption is not equally popular worldwide and most people neophobic to eat flowers. Night jasmine, Drumstick, Pumpkin and Nongmangkha flowers have been consumed traditionally since ancient times; not only as a food ingredient but also to cure many diseases. But the knowledge of edible flowers as food is mostly confined for traditional uses which hiders market viability. There are many such flowers which are edible, but due to lack of information or knowledge, people cannot see the novel use of these edible flowers. Many people are not consuming the flowers with the fear of presence of toxins. The scientific study or the data regarding the exploration and use of edible flower as food and safe consumption are scanty. Therefore, it is necessary to conduct scientific studies proposing flowers as health beneficial food to increase its awareness stimulate market growth and foster innovation in food technology. The aim of this research work is to evaluate the nutritional and mineral content, antioxidant activity and phenolic compounds of Night jasmine, Drumstick, Pumpkin and Nongmangkha flowers of Assam. Furthermore, there is limited exploration into the minerals and screening of phenolic compounds, making this study valuable for contributing and strengthening the existing literature of edible flowers or enhancing the current knowledge base on edible flowers. The findings will be helpful to promote edible flowers as valuable sources of nutrients which can be potentially advantageous for pharmaceutical and the food industry, and various other applications.

3.2. Materials and Methods

3.2.1. Sample collection

Flowers were collected during their full bloom season in the morning hours. Flowers of Night jasmine and Drumstick (Fig.3.1) were collected from Tezpur University campus, Napaam, Assam, India, and flowers of Pumpkin, Nongmangkha

(Fig.3.1) were collected from Betbari, Sivasagar, Assam, India. All these species of flowers were procured in the morning (6 am to 10 am) and transported to laboratory on the same day and kept in refrigerator at 4 °C. The collected flowers were then cleaned, trimmed, and washed with distilled water before drying them. Furthermore, the flowers were shade dried with blowing air (fan) and the dried flowers were ground, sieved and stored in an air tight container at refrigerated condition for further analysis.

(A)

(B)

(C)

(D)

Fig.3.1. Edible flower (A) Drumstick (B) Night jasmine (C) Nongmangkha and (D) Pumpkin

3.2.2. Moisture, ash, fat fiber and protein analysis

The dried flowers of Night jasmine, Drumstick, Pumpkin and Nongmangkha samples were analyzed for ash, fat, fiber and protein. Fresh flowers were taken for determining the moisture content by using standard AOAC method (AOAC, 2000). Socs plus (SCS6), Fibro plus (FES06) apparatus (Pelican Equipment, Chennai, India) were employed for determining total fat and total fiber of the flower samples respectively.

3.2.3. Extract preparation

The powdered flower (5 g each) samples was mixed with 50 mL of ethanol (80 %) and left 12 h in an orbital shaker at 27 °C. Afterwards, the samples were centrifuged at 6000 rpm for 30 min, and the supernatants were collected. The extracts were stored in the refrigerator at 4 ºC for further analysis of phytochemical and HPLC analysis of phenolic compounds. The obtained extract first concentrated in a rotary vacuum evaporator (ROTEVA-8703, Equitron, India) at 40 °C, and then further dried in a vacuum drier (Jeiotech, Korea, OV-11) at 40 °C.

3.2.4. Total phenolic content (TPC)

The determination of TPC of flower extract was performed by modification of Folin Ciocalteu method (Wetwitayaklung et al., 2007). Indeed, 500 μl of sample extract was mixed with 0.5 mL of diluted Folin Ciocalteu reagent followed shaking and after 3 min 20 % Sodium carbonate (Na_2CO_3) solution was added to it. The whole mixture was diluted to a total volume of 5 mL, mixed well using a vortex mixture and allowed to incubate for 1 h in the dark at room temperature. Finally, absorbance was read at a wavelength 760 nm using a spectrophotometer (Spectronic 20D+, Thermo Scientific, USA). Results were calculated based on mg gallic acid equivalent (GAE) per gram.

3.2.5. Total flavonoid content (TFC)

TFC was determined by the aluminum chloride colorimetric assay (Ammar et al., 2015). 250 μl of flower extract of Night jasmine (30 μl/mL), Drumstick (50 μl/mL), Pumpkin (50 μl/mL) and Nongmangkha (30 μl/mL) was mixed with 150 μl of 5 % $NaNO_2$ and after 6 min 75 μl 10 % $AlCl_3$ was added. After 5 min incubation at room temperature, there 1 mL of 1 M NaOH was added and total volume of the mixture was adjusted upto 10 mL with distilled water. Finally, the mixture was shaken in a vortex mixture and absorbance was measured at 510 nm using a spectrophotometer (Spectronic

20D+, Thermo Scientific, USA). Quercetin was used as the standard for a calibration curve and results were expressed as quercetin equivalent (QE) per gram.

3.2.6. DPPH radical scavenging activity

DPPH radical scavenging activity was determined using the method suggested by Tundis et al. (2015) with slight modifications. 0.5 mL sample extract was mixed with 2.5 mL of DPPH solution (0.1mM DPPH) and incubated for 30 min in dark at room temperature. The wavelength was read at 517 nm and percentage of DPPH radical scavenging activity was calculated (Eq. 3.1).

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \quad (3.1)$$

3.2.7. Ascorbic acid

Ascorbic acid content was determined by following the standard AOAC method (2000), extracting the flower sample in 4 % oxalic acid and titrating against the 2,6-dichlorophenolindophenol dye. The ascorbic acid content was calculated by using Eq. 3.2 and Eq. 3.3.

$$\text{Ascorbic acid} = \frac{Titrevalue \times Dyefactor \times Volumemadeup \times 100}{Aliquottaken \times Weightofthesample} \quad (3.2)$$

Dye factor (D.F) = 5.0/ titre value (3.3)

3.2.8. Minerals analysis by AAS

Sample digestion was performed by taking 1 g of dried flowers samples and adding them to a mixture of sulphuric and nitric acid (ratio 3:1) and digested to obtain a clear solution. After digestion, the samples were cooled and the volume was adjusted to 100 mL with deionized water. Finally, these digested samples were analyzed for minerals content such as potassium, sodium, iron, zinc, copper and manganese using Atomic Absorption Spectroscopy (AAS) (Thermo Scientific, Model Ice3500) with air and acetylene flame.

3.2.9. Cytotoxicity assay

Crude extract of flowers viz. Nongmangkha, Drumstick, Night Jasmine and Pumpkin were diluted to 1 mg/mL with deionized water and filtered it with 0.2 micronnylon filer. In this assay, HEK 293 cells (10,000 per well) were seeded into a 96-well plate. The cells were then treated with varying concentrations of flower extract (12.5, 25, 50, 100 & 200 µg/mL) for up to 24 h. After incubation, the cells were exposed to MTT and incubated for an additional 3 h. After that, the media was carefully removed, and MTT dissolving solution was introduced. The absorbance was measured at a wavelength of 590 nm using a UV-Vis spectrophotometer.

3.2.10. HPLC analysis

Phenolic compounds present in conventional extract of Nongmangkha, Night jasmine, Pumpkin and Drumstick flowers were detected by HPLC analysis. Binary RP-HPLC system (Waters, USA; 1525, 2414, 2489) gradient elution was performed. The phenolic compounds present in the flower extract were determined by comparing their retention time and UV–vis spectra with phytochemical analytical standards and detection was achieved ultraviolet detector and chromatograms were read at 280 nm and 360 nm. There were 14 phenolic compound standards were taken such as gallic acid, resorcinol, 3,4-dihydroxybenzoic acid, catechin, caffeic acid, syringic acid, rutin, salicyclic acid, ellagic acid, p-coumeric acid, quercetin, kaempherol, ferulic acid and apigenin. Dried flower extracts were taken and dissolved in HPLC grade methanol at a concentration of 1 mg/mL and then filtered with 0.2 µm Whatman syringe filter (nylon) in vials.

Based on Muchahary and Deka (2021), with slight modifications acidified Milli-Q water (1 % acetic acid, mobile phase A) and HPLC grade methanol (mobile phase B) were chosen as the mobile phases and flow rate was 1 mL/min for elution. With the help of glass syringe, 20 µl sample and standards were injected in the HPLC injector. The gradient mood was set as 0 min – 80 % A, 7 min- 80 % A, 10 min- 65 % A, 11 min- 65 % A, 16 min- 45 % A, 17 min- 45 % A, 24 min- 30 % A, 25 min- 30 % A, 30 min- 20 % A, 31min- 20 % A, 35 min- 10 % A, 36 min- 10 % A, 40 min- 80 % A. The quantification of phytochemicals was calculated from peak area with the respective standard calibration curves. All results were presented as milligram per gram (mg/g) dry extract.

3.2.11. Statistical Analysis

The experiment was examined by taking three replicates and the data obtained were subjected to statistical analysis by following one way analysis of variance (ANOVA) at a significance level $p \leq 0.05$.

3.3. Results and Discussion

3.3.1. Moisture, ash, fat, fiber and protein

The proximate compositions for all the flowers species were analyzed and are represented in Table 3.1. There was significant difference ($P < 0.05$) in the moisture content of all flowers in this study. Fresh flowers of Night jasmine, Drumstick, Pumpkin and Nongmangkha were showed to have moisture content from 92.76 % to 77.33 %. Night jasmine flowers showed the highest moisture content and the lowest moisture content was observed in Nongmangkha flowers. Ghosh and Rana (2021) also found 85.03 % moisture content in *Cucurbita maxima* flowers. In this study, Pumpkin flowers were found to have 82.48 % moisture content which was in line with the study by Ghosh and Rana (2021). It can be observed that all these 4 verities of flowers have different and high moisture content, which signifies the perishability of edible flowers.

Ash content was found to be in the range from 2.94 % (Night jasmine) to 6.551 % (Drumstick). Oyyeemi et al., (2017) also found ash content in flowers of *Hibiscus rosasinensis*, *Moringa oleifera* and *Musa paradisiacal* were 2.64 %, 1.89 % and 3.34 % respectively. Night jasmine and pumpkin flowers showed no significance difference ($P < 0.05$) in ash content. All the flowers showed more than 1 % of ash content and these findings also in line with other species of flowers i.e. *Spilanthes oleracea* (1.44 %) (Navarro-González et al., 2014), *Cucurbita maxima* (3.1 %) (Ghosh & Rana, 2021), and some flowers of *Zingiberaceae* family showed ash content of 1.46 % in *Alpinia galanga*, 1.21 % in *Curcuma plicata*, 1.66 % in *Zingiber officinale* etc. (Rachkeeree et al., 2018). Fat content found to be varied in all the four selected verities of flowers in this study which was ranges from 0.13 % (Drumstick) to 3.01 % (Pumpkin). There is a significant difference ($P < 0.05$) of fat content among the selected flowers. Rachkeeree et al. (2018) found fat content of 0.85 % and 0.61 %, in *Alpinia galangal* and *Amomum maximum* respectively. Also some other earlier reports on flowers reported to confer fat content of

5.0 g/100 g (Dry Weight) in *Cucurbita pepo* L. (Sotelo et al., 2007), *Moringa oleifera Lam*. 2.9 g/100 g (Dry Weight). It was observed that there is a significant difference ($P< 0.05$) of fiber content among the selected flowers. Nongmangkha flowers showed the highest amount of fiber content at 8.86 %, while the lowest was found to be 3.60 % in Night jasmine flower. *Alpinia galangal* reported to have 3.64 % of fiber (Rachkeeree et al., 2018). Along with biological structure of flowers also; soil quality, environmental difference etc. may influence the content of nutrients present flowers.

The ash and fiber content reported for *Premna latifolia* were 9.12 % and 15.30 % respctively; in *Amaranthus caudatus* were 24 % and 10.92 % and in *Lathyrus aphaca*were 8.49 % and fiber 13.61 % (Arasaretnam et al., 2018). Saikumar et al. (2023) reported that elephant apple powder has a crude fiber content of 20.58 %. These reported values are higher than those obtained values for the selected edible flowers in this study. In contrast, *Raphanus sativus* reported to have 0.79 % and 0.67 % and *Brassica rapa* had 0.63 % and 0.75 % ash and fiber content respectively (Rehman et al., 2014). These values are lower than those obtained values for the selected edible flowers.

Protein content was found to be in range of 10.15 % to 17 % among the selected flowers. It was observed that pumpkin flower had the highest content of protein and Nongmangkha flower has the lowest content of protein. There is no significant difference of protein content between Night jasmine and Nongmangkha flower. But Drumstick and Pumpkin flower showed the significant difference of protein content among the selected flowers. Earlier studies also reported that protein of 12.10 g 100 g^{-1} was observed in flower powder of Malvagoma (*Alcea rosea* L.) and 9.38 g 100 g^{-1} in Pena Pena (*Fuchsia hybrida***)** (Castillo-Carrión et al., 2024).

Table 3.1. Nutritional composition, phytochemicals, antioxidant activity and ascorbic acid contents of flowers of Night jasmine, Nongmangkha, Drumstick, Pumpkin

Composition	Night jasmine	Nongmangkha	Drumstick	Pumpkin
Moisture* (%)	92.76±2.68^{d}	77.33±2.63 ^{a}	84.74±2.10^{b}	87.86±2.47^{c}
Ash (%)	2.94±0.11^{a}	4.39±0.24 ^{b}	6.55±0.42 ^{c}	3.41±0.31^{a}
Fat (%)	2.56 ±0.37^{c}	1.36±0.27 ^{b}	0.13±0.04 ^{a}	3.01±0.09 ^{d}
Fiber (%)	3.60±0.24^{a}	8.86±0.50 ^{d}	6.69±0.09 ^{c}	5.03±0.19^{b}
Protein (%)	11.25±0.73^{a}	10.15±0.58^{a}	14.15±0.84^{b}	17.46±0.29^{c}
Ascorbic acid(mg/ 100 g)	0.051±0.005 ^{a}	0.137±0.036 ^{b}	0.042±0.004^{a}	0.049±0.002^{a}
TPC (mg GAE/g)	107.06 ±3.90^{c}	118.12±2.93^{d}	95.19±2.01^{b}	60.49±2.54^{a}
TFC (mg QE/g)	47.80±2.22^{c}	75.36±2.47^{d}	31.84±1.12^{a}	36.96±1.96^{b}
DPPH radical scavenging activity (%)	76.69±0.69^{b}	85.66±1.97^{c}	89.76±1.95^{d}	72.78±2.57^{a}

All data are the mean ± SD of three replicates. Mean followed by different letters in the same row differs significantly ($p \leq 0.05$). *Moisture content is reported for fresh flowers and other parameters are for dried flowers.

3.3.2. Ascorbic acid

In this study Drumstick, Pumpkin and Night jasmine flowers did not show any significant difference ($P<0.05$) in case of ascorbic acid content (Table 3.1). But ascorbic acid of Nongmangkha flower is significantly different from flowers of Drumstick, Pumpkin and Night jasmine. Nongmangkha flower showed the highest content of ascorbic acid i.e. 0.137 mg/100g. Ascorbic acid content was found in Night jasmine, Drumstick and Pumpkin flowers were 0.051 mg/100g, 0.042 mg/100g and 0.049 mg/100g respectively (Table 3.1). Rachkeeree et al. (2018) reported 0.08 mg/100 g ascorbic acid in *Curcuma plicata*. *Peliosanthes teta* flowers were also found to have 0.04 mg/100g ascorbic acid (Kantadoung et al., 2017).

3.3.3. Phytochemical content of edible flowers

Phenolic compounds are considered to provide various health benefits, have potential to reduce cancer risk and also have antioxidative properties. TPC and TFC values of Night jasmine, Nongmangkha, Drumstick and Pumpkin flowers expressed as mg GAE/g and mg QE/g respectively were shown in Table 3.1. There were significant differences (P< 0.05) of the total phenolic content found amongst the extracts of these studied flowers. Nongmangkha flowers had the highest content of total phenolic compound i.e. 118.12 mg GAE/g. Flowers of Night jasmine had 107.06 mg GAE/g, Drumstick had 95.19 mg GAE/g and Pumpkin showed 60.49 mg GAE/g of TPC. Regarding TFC, Nongmangkha flower showed the highest amount of TFC i.e. 75.36 mg QE/g. Furthermore, TFC was found in Night jasmine was 47.80 mg QE/g, Drumstick was 31.84 mg QE/g and in Pumpkin 36.96 mg QE/g. It was seen that there is a significant difference (P<0.05) in TFC among the selected flowers were seen among the selected flowers.

Malus pumila reported to have TPC of 60.58 mg GAE/g, in *Aglaia odorata* was 55.46 mg GAE/g (Zheng et al., 2018). Crude extract from *Pyrus pashia* flowers showed 108.8 mg GAE/g TPC (He et al., 2015). These results are in the line with TPC of selected flowers in our study. Kandyl is et al. (2022) reported *Rosa gallica* had 111.3 mg GAE/g of TPC in dry weight basis. Methanol extract of Opuntia *ficus indica L*. flowers showed 60.81 mg rutin equivalent/g of TFC (Ammar et al., 2015). In *Myrtillocactus geometrizans* 72.40 mg QE/g (Dry weight) TFC content was observed (Pinedo-Espinoza et al., 2020) which were almost similar with the TFC of Nongmangkha flowers of this study. Each plant extract had a higher total phenolic content compared to its total flavonoid content, due to the existence of non-flavonoid phenolic compounds in plants (Maisuthisakul et al., 2007; Pietta, 2000).

Other plant food such as *Alternanthera sessilis, Basella alba, Leucas cephalotes and Solanum nigrum* report to have TPC of 292.65, 72.66, 164.96 and 97.96 mg GAE/g dry extract respectively and TFC were 21.51, 6.97, 36.95, 16.42 mg QE/g dry extract respectively (Aryal et al., 2019). The selected edible flowers contains higher amount of TFC compared to these samples. However, *Alternanthera sessilis* and *Leucas cephalotes* contains higher amount of TPC compared to studied edible flowers.

3.3.4. DPPH radical scavenging activity

There is a significant difference (P< 0.05) in percentage of DPPH radical scavenging activity among the selected flowers (Table 3.1). Drumstick flower found to have the highest percentage of DPPH radical scavenging activity i.e, 89.76 %. After that percentage of DPPH radical scavenging activity obtained 85.66 % in Nongmangkha, 72.78 % in pumpkin and 76.69 % in Night jasmine. Earlier study reported that *Antigonon leptopus* showed 89.36 % and *Plumeria obtuse* 69.65 % of DPPH radical scavenging activity (Kaisoon et al., 2011). The effectiveness of the extracts in scavenging radicals varied among the flowers. There might be various reasons for this but also one reason is the total amount of phenolic compounds present in them. Indeed, the antioxidant efficacy of phenolic compounds depends on the quantity and positioning of hydroxyl groups, as well as the presence of electron-donating substituents within the ring structure (Elzaawely et al., 2007; Lapornik et al., 2005).

3.3.5. Mineral content of edible flowers

Understanding the mineral composition of flowers is crucial for assessing their nutritional value and ensuring they are safe for human consumption. An inadequate availability of essential minerals could lead to numerous deficiency illnesses and hinder growth. Some minerals (Cadmium, Lead, Mercury, and Arsenic etc.) could provide negative health impacts as they are listed as toxic. Mineral content of edible flowers were determined by flame atomic absorption spectroscopy, where potassium, sodium, iron, zinc, copper and manganese were quantified (Table 3.2). Potassium found to be the most abundant mineral in these flowers. Pumpkin flowers have the highest amount of potassium content i.e. 14.412 mg/g. Flowers of Night jasmine, Drumstick, and Nongmangkha were showed potassium content of 11.956, 12.912 and 12.401 mg/g respectively. Earlier study by Oyeyemi et al. (2017) reported the potassium content of 4.3755 mg/g in *Moringa oleifera*, 4.0481 mg/g in *Hibiscus rosasinens* is and 3.9517 mg/g in *Musa paradisiacal.* Also the potassium content in *Calendula officinalis* was reported 2.98864 mg/g (Mlcek et al., 2021) and 5.89 mg/g in *Alpinia galangal* (Rachkeeree et al., 2018).

Table 3.2. Minerals content (mg/g) in Night jasmine, Nongmangkha, Drumstick, Pumpkin flowers

Sl. no.	Minerals (mg/g)	Night jasmine	Nongmangkha	Pumpkin	Drumstick
1	Potassium	11.956	12.401	14.412	12.912
2	Sodium	0.768	0.486	0.376	0.523
3	Iron	0.513	0.341	0.254	0.235
4	Zinc	0.069	0.046	0.060	0.130
5	Copper	0.047	0.010	0.017	0.009
6	Manganese	0.009	0.014	0.042	0.009

Night jasmine flowers showed the highest content of sodium content (0.768 mg/g) among the studied flowers. Sodium content was obtained as, 0.523 mg/g in Drumstick, 0.376 mg/g in Pumpkin and 0.486 mg/g in Nongmankha. Araújo et al. (2019) reported to have sodium content in flowers of *Lavandula angustifolia*, *Lonicera japonica*, *Rosa sp*. and *Rosmarinus officinalis* were 0.752, 0.859, 0.795 and 0.725 mg/g respectively.

Iron content was found highest in Night jasmine flower (0.513 mg/g) among all the flowers in this study. Iron content 0.341 mg/g in Nongmangkha, 0.254 mg/g in Pumpkin and 0.235 mg/g in Drumstick flower were also obtained. It is seen that iron content varies among species to species. Similarly, earlier report found iron content of 0.3488 mg/g in *Hibiscus rosasinensis*, 0.3531 mg/g in *Moringa oleifera* and 0.4376 mg/g in *Musa paradisiacal* (Oyeyemi et al., 2017). Zinc content was found highest in Drumstick flower i.e. 0.130 mg/g among all the flowers in this study, which is in line with zinc content of *Rosa sp* (0.116 mg/g) reported by Araújo et al. (2019). Flowers of Night jasmine, Pumpkin and Nongmangkha were found to have zinc content of 0.069 mg/g, 0.060 mg/g and 0.046 mg/g. *Calendula officinalis* reported to have 0.0603 mg/g of zinc (Araújo et al., 2019).

Copper content was obtained highest i.e. 0.047 mg/g in Night jasmine. 0.009 mg/g copper content was found in Drumstick, 0.017 mg/g in Pumpkin and 0.010 mg/g in Nongmangkha. Manganese was found lesser than the potassium, sodium, iron and zinc among flowers of Night jasmine, Nongmangkha, Drumstick and Pumpkin. Manganese was resulted to have 0.009 mg/g in both Night jasmine and Drumstick flowers, 0.042 mg/g in Pumpkin flower and 0.014 mg/g in Nongmangkha flower. Oyeyemi et al. (2017) were found zinc, copper in *Hibiscus (Rosa sinensis)* were 0.1302 mg/g, 0.0386 mg/g, in *Moringa oleifera* were 0.1125 mg/g, 0.0404 mg/g and 0.1574 mg/g of manganese and *Musa paradisiacal* were 0.1186 mg/g, 0.0414 mg/g respectively and 0.2867 mg/g of manganese. It was seen that minerals present in all the species of flowers are different. 0.0099 mg/g and 0.006 mg/g of manganese were reported in *Antirrhinum majus L.* and *Paeonia officinalis L.* (Grzeszczuk et al., 2018). The mineral makeup of flowers is impacted by various factors such as natural absorption of minerals from the soil and environment (Araújo et al., 2019). However, these results were signified that the edible flowers could be a good natural source of minerals.

3.3.6. Cytotoxicity in HEK-293 cells

To evaluate the cytotoxicity effect of edible flowers extract viz. Nongmangkha, Night jasmine, Pumpkin and Drumstick on HEK-293 cells, the cell lines were treated with various concentration of flower extract. The cytotoxicity was observed to be dose dependent (Fig.3.2) (Table 3.3). The cell viability was observed to be different on the basis of the species of flowers. Cell viability of Nongmangkha flower extract was found to be 82.62 % at extract concentration of 200 µg/mL for 24 h. Among all these flower extract, Pumpkin flower showed to offer highest cell viability of 93.36 % at extract concentration of 200 µg/mL for 24 h.

Table 3.3. Cell viability of HEK-293 cells of edible flower extract

Concentration of extract (µg/mL)	Nongmangkha (%)	Night jasmine (%)	Pumpkin (%)	Drumstick (%)
Control	100	100	100	100
12.5	95.92±5.91	97.15 ±4.51	100.01±6.18	98.84±4.33
25	95.02 ±5.23	96.09 ±5.07	99.17±6.72	95.71±6.35
50	90.63 ±6.35	93.97 ±5.57	96.4±5.01	94.37±5.71
100	88.55 ±4.87	90.43 ±3.03	96.22±2.55	92.08±6.97
200	82.62 ±7.13	88.29 ±3.28	93.36±6.59	86.93±1.35

All the data are average of three replicates and ± standard deviation

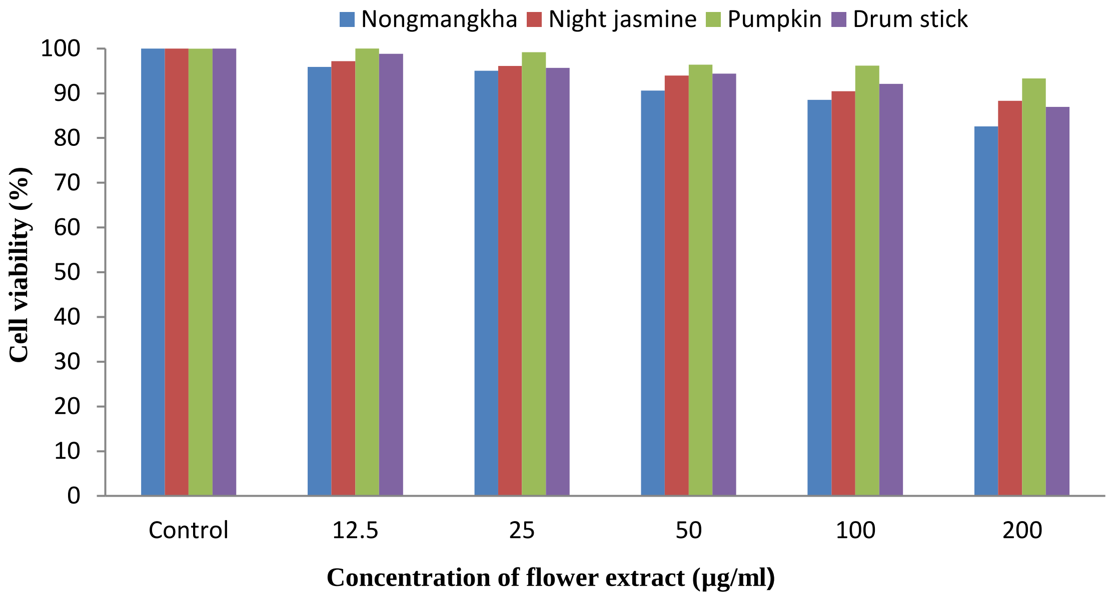

Fig.3.2. Cell viability results of various edible flower extracts on HEK-293 cells

3.3.7. Phenolic compounds detected by HPLC in edible flowers' extract

In this study, standards of phenolic compounds were introduced into the HPLC system and found to vary across all the selected flowers such as Nongmangkha, Night Jasmine, Drumstick, and Pumpkin (Table 3.4). HPLC chromatograms of phenolic compounds of flowers are represented in Fig.3.3. To analyze the data initially the retention time of each peak was compared with the standards of phenolic compounds. Night jasmine flowers were found to confer gallic acid (4.612 mg/g), resorcinol (4.161 mg/g), caffeic acid (0.005 mg/g), p-coumeric acid (14.187 mg/g), ferulic acid (7.827 mg/g). Pumpkin flowers were observed to detect only resorcinol (4.525 mg/g), catechin (1.313 mg/g) and quercetin (1.987 mg/g). Nongmangkha flowers were observed to have resorcinol (3.813 mg/g), 3, 4-dihydroxybenzoic acid (1.851 mg/g), rutin (8.142 mg/g), ellagic acid (4.112 mg/g), salicyclic acid (20.729 mg/g), kaempherol (0.386 mg/g) and apigenin (0.387 mg/g). Drumstick flowers found to detect only ellagic acid (4.122 mg/g), salicyclic acid (10.470 mg/g) and apigenin (0.085 mg/g). It was observed that all the selected species of flowers have different phenolic compounds. Based on the 14 HPLC-grade phenolic compound standards used in this study, it was observed that Nongmangkha and Night jasmine flowers exhibited more of these phenolic compounds compared to Pumpkin and Drumstick flowers. The observed unknown peaks paved a way to investigate more about phenolic as well as other phytochemical compounds and these unknown compounds may also support the antioxidant activity of flowers.

Table 3.4. Phenolic compounds detected by HPLC in edible flowers' extract

Sl no.	Phenolic compound	Night jasmine (mg/g)	Pumpkin (mg/g)	Drumstick (mg/g)	Nongmangkha (mg/g)
1	Gallic acid	4.612	Not detected	Not detected	Not detected
2	Resorcinol	4.161	4.525	Not detected	3.813
3	Caffeic acid	0.005	Not detected	Not detected	Not detected
4	Catechin	Not detected	1.313	Not detected	Not detected
5	3,4 dihydroxy benzoic acid	Not detected	Not detected	Not detected	1.851
6	p-coumeric acid	14.187	Not detected	Not detected	Not detected
7	Syringic acid	Not detected	Not detected	Not detected	Not detected
8	Rutin	Not detected	Not detected	Not detected	8.142
9	Ellagic acid	Not detected	Not detected	4.122	4.112
10	Salicyclic acid	Not detected	Not detected	10.470	20.729
11	Quercetin	Not detected	1.987	Not detected	Not detected
12	Ferulic acid	7.827	Not detected	Not detected	Not detected
13	Kaempferol	Not detected	Not detected	Not detected	0.386
14	Apigenin	Not detected	Not detected	0.085	0.387

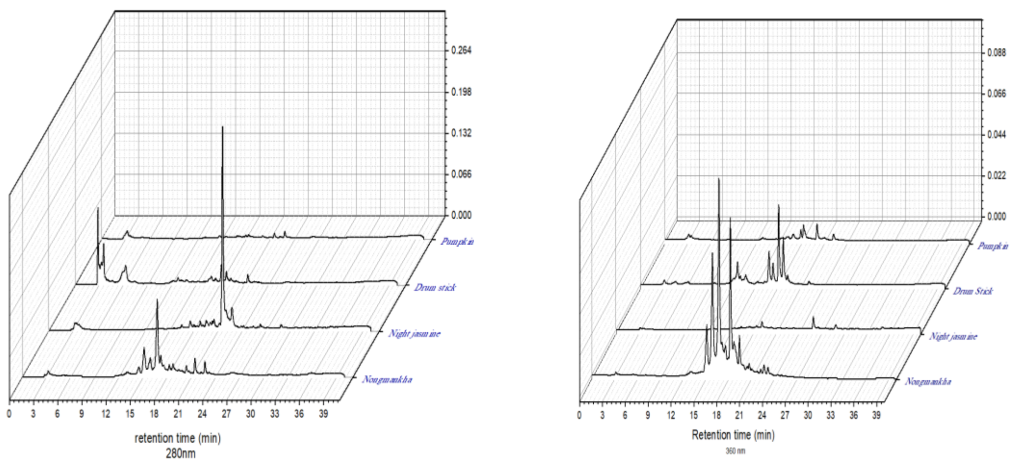

Fig. 3.3. RP-HPLC Chromatograms of phenolic compounds of Nongmangkha, Night jasmine, Drumstick and Pumpkin flower at 280 nm and 360 nm.

3.4. Conclusions

This study provides important insight into composition and indicates the presence of various nutritional compounds, minerals, phenolic compounds and antioxidant activity of the investigated edible flowers i.e. Night jasmine, Nongmangkha, Pumpkin and Drumstick. It was observed that every flower differs from each other regarding the contribution of nutrients both qualitatively and quantitatively. Moisture content was found in them in the range from 77.33 % to 92.76 %, which indicates the perishability of edible flowers. Drumstick flower showed the highest amount of ash content (6.55 %) and pumpkin flower had the highest amount of fat content (3.01 %) and protein content (17.46 %). Highest content of fiber i.e. 8.86 % was observed in Nongmangkha flower. Mineral analysis revealed the presence of potassium, sodium, iron, zinc, copper, and manganese in varying concentrations in the selected edible flowers, with potassium being the most abundant mineral among them. Here, TPC was found in the selected edible flowers ranged from 60.49 to 118.12 mg GAE/g and TFC ranged from 31.84 to 75.36 mg QE/g. Furthermore, antioxidant activity was found in the range from 72.78 to 89.76 %. HPLC analysis was used to explore the phenolic compounds in them, where 14 different phenolic compound standards were taken. Results showed that these edible flowers contain various phenolic compounds which vary in concentrations. HPLC analysis for the screening of phenolic compounds resulted that these edible flowers contain various classes of phenolic compounds which vary in concentrations. Potassium found to be the most abundant minerals in these flowers as compared to the sodium, iron, zinc, copper and manganese. The results indicated that these edible flowers could be a natural source of nutrients, mineral, phenolic compounds etc.

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