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# Comparative analysis of antioxidant activities of *Vitex negundo* and *Ficus carica* leaf extracts

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DPPH assay

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**ABSTRACT**

Humans have been dependent on nature for various vital supplies and resources for a long time. Most biotechnological and pharmacological industries use chemicals and active compounds to treat diseases or make medications isolated from natural resources. A variety of plants have been explored for research of which *Vitex negundo* and *Ficus carica* are also examples as they are strong candidates for their potential antioxidant properties. In the current research, the anti-oxidant activities of *V. negundo* and *F. carica* leaf extracts were evaluated. The antioxidant activities of selected plants were analyzed using DPPH and FRAP assay. The results obtained from the DPPH assay indicated that methanolic extracts of *V. negundo* showed the highest inhibition of 90.07±1.17 percent at 1000 µl with $IC_{50}$ value of 415.98 µg/ml followed by ethyl acetate and chloroform extracts (64.05±0.89 and 54.39±0.99 percent, respectively) with $IC_{50}$ value of 751.96 µg/ml and 896.55 µg/ml when compared to *F. carica* extracts which showed highest inhibition of 75.75±1.08 percent at 1000 µl with $IC_{50}$ value of 475 µg/ml followed by ethyl acetate and chloroform extracts (51.94±0.79 and 44.21±0.60 percent respectively) with $IC_{50}$ value of 967.51 µg/ml and 1092.48 µg/ml. On comparing both plants, FRAP results indicated that methanol extracts of *V. negundo* showed the highest FRAP value (1042.1±0.98 µM) followed by ethyl acetate and chloroform extracts, which shows 996.6±1.25 µM and 949.6±1.63 µM at 1000 µl whereas *F. carica* showed highest FRAP value (995.6±1.35µM) followed by ethyl acetate and chloroform extracts, which shows 987.6±1.05µM and 447.6±1.01µM at 1000 µl. The results of the study can be concluded that among the tested extracts, the best antioxidant potential was exhibited with *V. negundo* leaf extracts, especially in methanol extracts.

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## 1 Introduction

Oxidative stress is a normal phenomenon and one of the most concerning elements in the scientific community. Oxidative stress arises when the number of oxidants increases in the body above normal level while the amount of antioxidants in the body decreases, resulting when free radicals are produced (Rahal et al. 2014). Free radicals have received a lot of attention. As part of standard cellular function, free radicals are produced continuously in all cells. Abundant free radical production from endogenous or exogenous sources could be a factor in the development of many diseases. Antioxidants play a significant role in the maintenance of tissue health (Young and Woodside 2001).

An antioxidant prevents reactive oxygen species (ROS) formation and helps the biological system sustain improved health against numerous diseases, including inflammation, liver damage, and cardiovascular disease that can be spread by these reactive oxygen species (Liao and Yin 2000). Antioxidants are frequently used as food supplements to prevent food deterioration. Prior studies have demonstrated that foods that have high antioxidants play a critical role in lowering the chance of developing heart illnesses, as well as other chronic diseases (Mata et al. 2007). Because several lifestyle-related diseases and the aging process are intimately linked to active $O_2$ and LPO, anti-oxidants play a crucial role in preventing these conditions (Noguchi and Niki 2019).

Earlier, synthetic anti-oxidants made up through chemical processes were utilized in many food and pharmacology industries but had various negative effects on human health. Therefore, a natural way of antioxidant preparation was opted for through the use of natural sources such as plant extracts to reduce the toxic and negative impact of synthetic antioxidants. Plants consist of many valuable bioactive compounds and are a perfect candidate for antioxidant production. Most of the anti-oxidants are derived from plant materials such as fruits, vegetables, herbs, and leaves (Hasani et al. 2007). There have already been numerous plant species evaluated for possible anti-oxidant properties (Hussain et al. 2008). *Vitex negundo* and *Ficus carica* have been used as food products and traditional medicines for the treatment of various diseases.

*V. negundo* Linn. belongs to the family Verbenaceae, it has quadrangular branches and tri- or penta-foliate leaves with 5 leaflets grouped like a palm and so is also known as the 5-Leaved Chaste Tree. Further in Indian traditional medicine, *V. negundo* is known as "sarvaroganivarani" which means "the remedy for all diseases" (Sabbagh and Kim 2022). The plant prefers to moisten environments to grow that's why it preferred to grow in India, Thailand, Madagascar, Malaysia, Sri Lanka, Eastern Africa, and Pakistan. Each part of the plant is developed with medicinal value; hence this plant plays a crucial role in traditional medication systems. Since the plant has therapeutic potential in every component, it is essential in systems of traditional medicine (Tandon 2005). All parts of *V. negundo* contain several phytoconstituents like fatty acids, alkaloids, flavonoids, phenols, glycosidicirridoids, lignans, tannins, steroids, and di- and sesquiterpenes. Due to the presence of a variety of secondary metabolites, *V. negundo* is used to treat different types of diseases such as spermatorrhoea, stomachache, asthma, cold, diarrhoea, indigestion, gallstone, hernia, eye disorders, rheumatism, irritable bladder and dysmenorrhea, headache, migraine, kwashiorkor, neck gland sores, tubercular neck swelling, reddened, arthritis, jaundice, urticaria, eczema, and liver disorders. It is most widely used for curing disorders of the reproductive system like vital power, frail erection without libido, stool-containing prostatic fluid, and testicle pain (Perveen et al. 2023). In Unani medicine, the seeds of *V. negundo* are also utilized as an aphrodisiac and to treat swellings. Chinese medicine recommends consuming the fruit of the *V. negundo* plant to alleviate headaches, soreness, and swollen eyes (Liu et al. 2005).

Over 800 species of the incredibly vast pantropical genus Ficus (family Moraceae) can be found globally (Adhikari et al. 2023). *Ficus carica* Linn. also known as the "common fig" or "Anjeer," is one of them. The leaves have an oval shape, a pubescent underside, a rough top, and three to five lobes (Taviano et al. 2018). Extracts from the roots, barks, leaves, and fruits, have a variety of pharmacological properties, including anti-inflammatory, anti-diabetic, antioxidant, anti-inflammatory, anti-arthritic, anti-hyperlipidemic, and gastroprotective, effects (Adhikari et al. 2023). The biological activities of *F. carica* are mostly related to the presence of diverse phytoconstituents present in the roots, latex, leaves, and fruits including anthocyanins, organic acids, amino acids, phytosterols, aliphatic alcohols, fatty acids, and phenolics (Li et al. 2021). *F. carica* boosts total protein expression, notably for genes relevant to fertility, and possesses anti-hyperglycemic properties (Bakar et al. 2020). *F. carica* leaves are consumed as a tea or utilized as a medication (Barolo et al. 2014). According to reports, *F. carica* leaves are helpful in several conditions like pustules, hemorrhoids, diabetes, dysentery, breathing, heart, and skin problems (Taviano et al. 2018).

Both *V. negundo* and *F. carica* have been reported to possess various medicinal values but despite this, no detailed work is reported. Therefore, in this study anti-oxidant potential of methanol, ethyl acetate, as well as chloroform leaf extracts of both *V. negundo* and *F. carica* plants have been evaluated.

## 2 Materials and Methods

### 2.1 Plant materials

The experimental plant part i.e., *V. negundo* (nirgundi) and *F. carica* (Anjeer) fresh leaves were collected from the Neem Vatika

Figure 1 NeemVatika Herbal Park, Samargopalpur, Rohtak, Haryana

Herbal Park, Samargopalpur, Rohtak, Haryana, followed by their proper authentication was done.

**2.2 Preparation of Extracts**

Leaves of *V. negundo* and *F. carica* were properly cleaned two to three times under running water, and then air dried at 32°C to 37°C in a shady place for about 2-3 weeks. The dried plant samples were ground into powder form by using a homogenizer. After that, 50 grams of dried and coarsely powdered plant leaf samples were extracted with solvents $CHCl_3$ followed by $C_4H_8O_2$ and $CH_3OH$ in order of their increasing polarity for the sequential extraction using the Soxhlet apparatus. These extracts were filtered individually and concentrated as dried mass for further use (Gupta 2005).

**2.3 Determination of antioxidant activities**

**2.3.1 DPPH radical scavenging activity**

The modified Brand-Williams et al. (1995) method was employed to analyze DPPH. For this, 1 mg/ml samples were prepared in methanol individually. The range of 100-1000 µl of the sample was selected and volume was maintained up to 1 ml with methanol. 1 ml of the DPPH solution (1 mg/10 ml) was added to all test tubes and vortexed. Tubes were then placed in the dark surrounding for thirty minutes. Later, the sample was replaced with methanol in the blank and absorbance was taken at 517 nm. Ascorbic acid was used as standard. To calculate the DPPH radical scavenging activity the following formula was used:

$$\% \text{ inhibition of DPPH} = \{(A_B - A_S)/A_B\} \times 100$$

Here, $A_S$ = absorbance of samples; $A_B$ = absorbance of blank or reference.

**2.3.2 Ferric reducing antioxidant power (FRAP)**

FRAP assays were conducted by using Pulido et al. (2000) method. The sample range of 100 µl to 1000 µl was fixed for FRAP estimation. During standardization, an aqueous solution with known $Fe^{2+}$ content (10 µM) was used in the range between 100–1000 µmol/l. Methanol was used for volume make-up of up to 1 ml. In this, 1 ml FRAP reagent was added in each sample and standards tubes and vortexed properly then incubated for 30 minutes at 37°C before using. The absorbance observations were recorded at 593 nanometres. The blank was made without any sample addition and the quercetin was taken as standard. By contrasting the activities of the standard curve, the proportional activities of the samples were evaluated. The results were expressed in a micromolar.

**2.4 Statistical Analysis**

These results from trials that were carried out in triplicates were noted as mean ± SD.

**3 Results and Discussion**

**3.1 DPPH assay**

The ability of diverse samples, including plant extracts, to scavenge free radicals is frequently assessed using the scavenging of the stable DPPH radical. Each extract ($CHCl_3$, EtOAc, and $CH_3OH$) was tested for DPPH scavenging activity in the current investigation, and it was found to be rising in a dosage-dependent pattern. The highest concentration of MeOH extract (1000µl) demonstrated the highest anti-oxidant activity for both plants (90.07±1.17% in *V. negundo* and 75.75±1.08% in *F. carica*), followed by EtOAc (64.05±0.89 % and 51.94±0.79 %) and $CHCl_3$ extract (54.39±0.99% and 44.21±0.60%), as shown in Figure 2 and 3. Additionally, the MeOH extract of *V. negundo* showed the

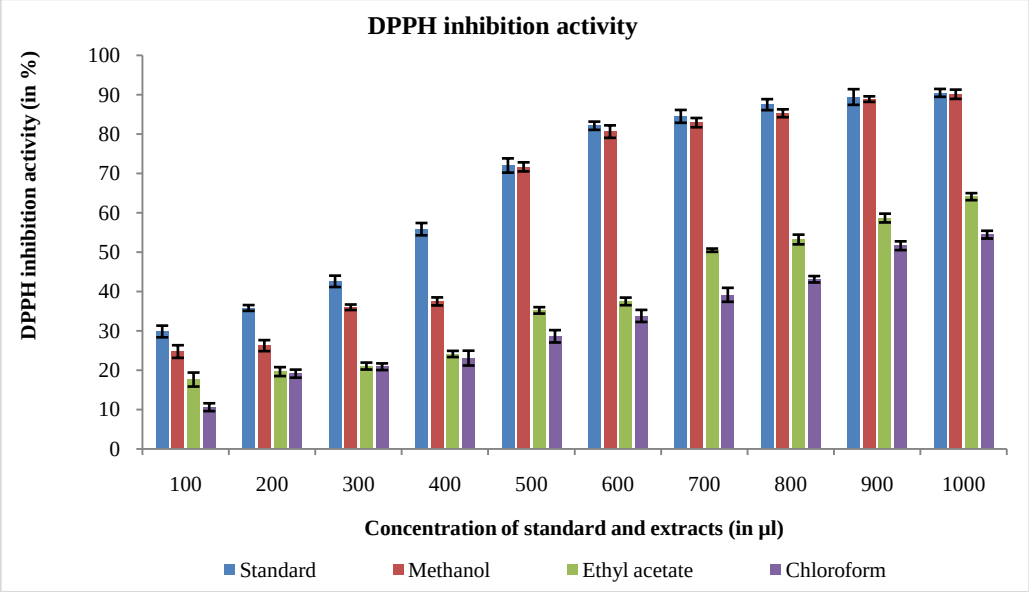


Figure 2 DPPH inhibition activity of *V. negundo*

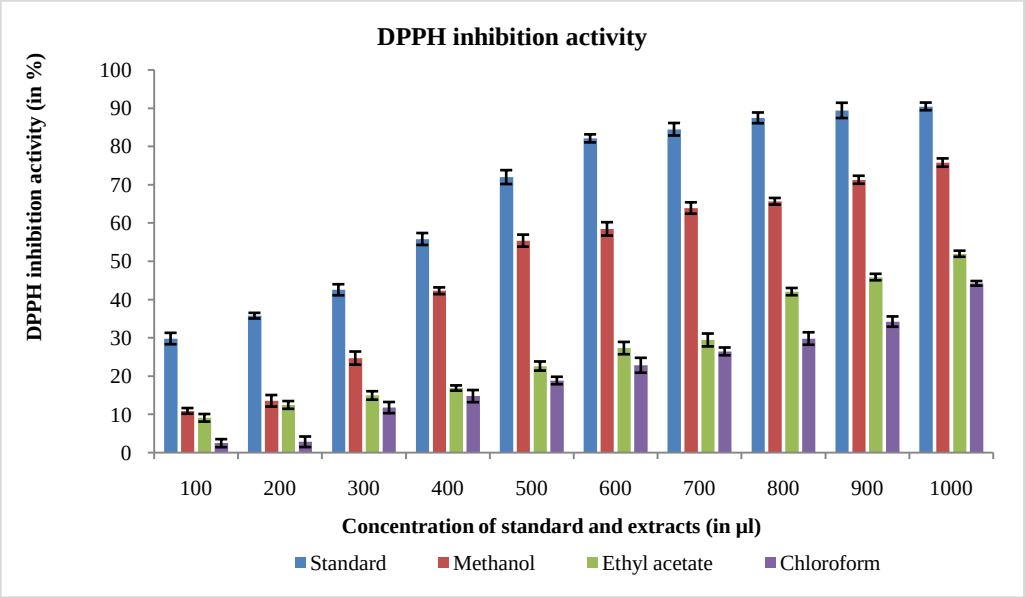


Figure 3 DPPH inhibition activity of *F. carica*

Table 1 $IC_{50}$ values of *V. negundo* and *F. carcica*

| Plant Samples | $IC_{50}$ (µg/ml) | | | |
|---|---|---|---|---|
| | Standard (Ascorbic acid) | Methanol | Ethyl acetate | Chloroform |
| *V. negundo* | 360 ± 1.58 | 415.98 ± 0.76 | 751.96 ± 1.32 | 896.55 ± 2.76 |
| *F. carcica* | | 475 ± 1.78 | 967.51 ± 2.34 | 1092.48 ± 3.22 |

comparatively best minimum inhibition activity ($IC_{50}$) (Table 1). When compared to the extracts from *F. carica*, it was shown that the extracts from *V. negundo* had excellent DPPH inhibitory activity and a remarkable $IC_{50}$ value. Similar to this, Ahmad et al. (2013) experiment showed that the anti-oxidant property of the *F. carica* leaves extract dramatically rose to the extract concentration.

At 25 µg/ml of sample, scavenging inhibition was observed 1.285 ± 0.15 % while at 250 µg/ml, it was observed 1.00 ± 0.09%. Anti-oxidants are thought to affect DPPH because of their capacity to donate hydrogen. It was clear that the extracts did exhibit some proton-donating potential and might act as free radical inhibitors or scavengers, possibly acting as major anti-oxidants, even though their DPPH radical scavenging activities were substantially lesser than those of ascorbic acid. Another investigation on the antioxidant potential of latex from unripe fruits of *F. carica* cultivar Pingo de Mel (Northeast Portugal) was carried out by Oliveira et al. (2010) who reported the $IC_{25}$ value for the DPPH test was 1049µg/ml. Further, the latex may also inhibit the development of additional biologically significant oxidative species, such as peroxynitrite and OH radicals, as a result of the interaction between these two oxidizing agents, based on the demonstrated scavenging capacity of the latex. To assess the *V. negundo* ethanolic extract's capacity to serve as hydrogen atom or electron donors in the transformation of the DPPH radical into its reduced form DPPH-H, Kadir et al. (2013) used the DPPH test. The stable, purple-colored radical DPPH could be converted by the extract of *V. negundo* into the yellow-colored DPPH-H. When DPPH radical scavenging activities of *V. negundo* extract, gallic acid, BHT, and ascorbic acid were compared, the percentage of radical scavenging activity for *V. negundo* was 79.43 ± 1.3 %, BHT was 82.53 ± 1.7 %, gallic acid was 89.51 ± 1.14 %, and ascorbic acid was 90.65 ± 1.34% at the highest concentration. These results suggested that *V. negundo* exhibited notable DPPH inhibitory action. Pinipay et al. (2022) also studied the effect of *F. religiosa* seed extracts on the percentage of DPPH inhibitions and reported that the chloroform extract had the highest percentage of inhibition (68.97 ± 0.08), which is nearer to that of standard ascorbic acid (75.60 ± 0.03), and BHT (69.30 ± 0.15). Further, hexane, chloroform, ethyl acetate, methanol, and aqueous extracts of *F. religiosa* had $IC_{50}$ values for DPPH activity was 140.25±11.22 g/ml, 131.17±0.41 µg/ml, 148.78±0.92 µg/ml, 143.87±10.37 µg/ml, and 116.52 ±2.74 µg/ml respectively. Teruel-Andreu et al. (2023) examined the leaves of four biferous varieties of *F. carica*: San Antonio (SA), Colar (CA), CuelloDamaNegra (CDN), and Superfig (SF) and observed DPPH assay between 72.45 - 52.54 mMTroloxdw. Furthermore, Ginting et al. (2020) assessed the percentage of DPPH scavenging activity of quercitrin, morin, myricitrin, and eleutheroside, as well as the ethanolic extract of *F. elastic* (FEE). When compared to other compounds (eleutheroside B, morin, quercitrin, and myricitrin), FEE had the least attribute at the greatest concentration (31.26 µg/ml), with a value of 62.52±0.66 %. Comparing other compounds, FEE displayed the highest $IC_{50}$ value (13.82±0.51 µg/ml), indicating that it has the lowest DPPH scavenging activity. Similarly, Le et al. (2022) reported that 80% ethanol extracts of *V. rotundifolia* showed greater radical scavenging activity than those of 100% MeOH extracts at concentrations of 10 and 100 µg/mL. In the current investigation, leaf extracts from *V. negundo* had the highest DPPH inhibitory activity when compared to leaf extracts from *F. carica*.

### 3.2 Ferric reducing antioxidant power (FRAP) assay

The ferric-reducing antioxidant power test evaluates the potential of an anti-oxidant compound and its reduction from $Fe^{3+}$-TPTZ to $Fe^{2+}$-TPTZ. The result of the present study depicted that the anti-oxidant potential of MeOH extract was significantly higher than that of $C_4H_8O_2$ and $CHCl_3$ extracts respectively for leaves of *F. carica* and *V. negundo* with the former being more efficient. But as compared to the *F. carica* extracts, *V. negundo* extracts showed higher FRAP values as shown in Figures 4 and 5. In the FRAP assay conducted by Soni et al. (2014), the vivid blue color that results from the reduction of the $C_{18}H_9FeN_6$ (ferric tripyridyltriazine) to $Fe^{2+}$was observed at a wavelength of 593 nm. FRAP activity was found to be very good in *F. carica* extract (60.48 µM). Another antioxidant study (FRAP) conducted by Vijayalakshmi and Rao (2020) on *V. negundo* revealed that water extract (90.56 µM) had greater antioxidant potential than quercetin (85.162 µM) and ascorbic acid (79.647µM). The antioxidant activity of the $C_4H_8O_2$ (80.67µM) and $CH_3OH$ (84.57µM) extracts were likewise positive and were closer to the ranges of the standards. Similarly, the result of Zargar et al. (2011) revealed that the MeOH extract of *V. negundo* has an antioxidant capacity of 44.6 ± 7.8 µM TE/g which was significantly higher than that of the plant's essential oil (11.53±1.35 µM TE/g) and hexane (11.30±1.3 µM TE/g) leaf extracts. Nevertheless, it was discovered that $CH_3OH$ extract's antioxidant capacity was four times greater compared to essential oil as well as hexane extract. Likewise, in another study by Traore et al. (2021), the fruit extracts of *V. doniana* differed in their ability to reduce $Fe^{3+}$, although all results were below standard i.e. butylated hydroxytoluene and ascorbic acid. Ayoub et al. (2019) analyzed the reducing capabilities of extracts from *F. carica* as well as *O. europaea*, and the results revealed that extracts had a strong reduction power and it was dosage dependent and increased with the levels of extract. Results of the study revealed that *O. europaea* extract had a FRAP value that ranged from 0.125±0.001 to 0.683 ±0.026 µg/ml, while this value was reported from 0.113 ± 0.004 to 0.494 ± 0.008 µg/ml and 0.260± 0.014 to 2.81± 0.014 g/ml for *F. carica* and ascorbic acids respectively. Therefore a prominent statistical difference was reported in these. *F. carica* leaves of the four biferous variants i.e. San Antonio, Colar, Cuello Dama Negra, and Superfig were examined for FRAP by Teruel-Andreu et al. (2023). The cultivars were ranked from top to lowest in terms of MTroloxdw: CDN > SF > CUMH > CA > SA with values being 124.79, 115.66, 67.15, 60.70, and 56.09 mMTroloxdw respectively. The FRAP test of *V. doniana* fruit indicated a value of 600.19 ± 2.37 µM as reported by Moffo Foning et al. (2022). In the study of Ginting et

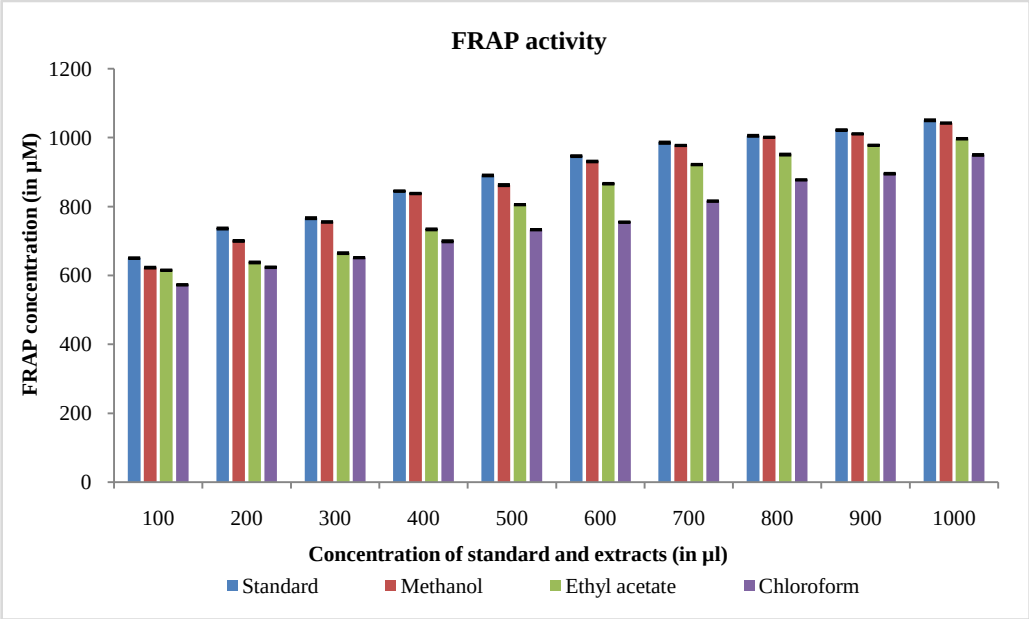


Figure 4 FRAP activity of *V. negundo*

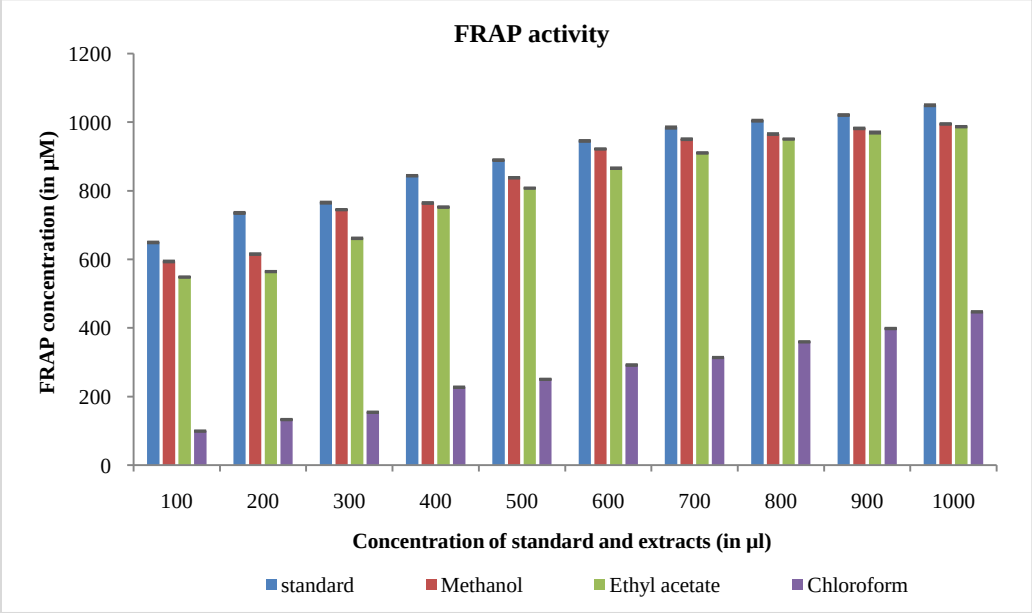


Figure 5 FRAP activity of *F. carica*

al. (2020), it was discovered that compounds, including eleutheroside B, quercitrin, morin, and the ethanolic extract of *F. elastica*, had FRAP-reducing action. At the maximum concentration (50.00 µg/mL), ethanolic extract demonstrated the least amount of FRAP-reducing action in comparison to eleutheroside B (117.08±27.35 µM Fe (II)/µg), myricitrin (456.00±13.43 µM Fe (II)/µg), quercitrin (487.58±5.59 µM Fe (II)/µg), and morin (496.58±9.25 µM Fe (II)/ µg). As a result, the ethanolic extract has a moderate level of antioxidant activity that is comparable to that of the morin molecule. Likewise, in the current study, the three *F. carica* leaf extracts gave FRAP activity, while the three *V. negundo* extracts displayed the highest FRAP activity.

## Conclusion

Results of the current research revealed a comparative analysis of the antioxidant activities of *V. negundo* and *F. carica* leaves extracts. Based on the findings, it can be concluded that the

methanolic extract of *V. negundo* possesses more antioxidant activity as compared to the *F. carica* extracts. However, further investigations for potential applications and *in vivo* experiments, are needed to verify these antioxidant effects of the selected plant species.

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**Conflicts of Interest**

The authors declare no conflict of interest.

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