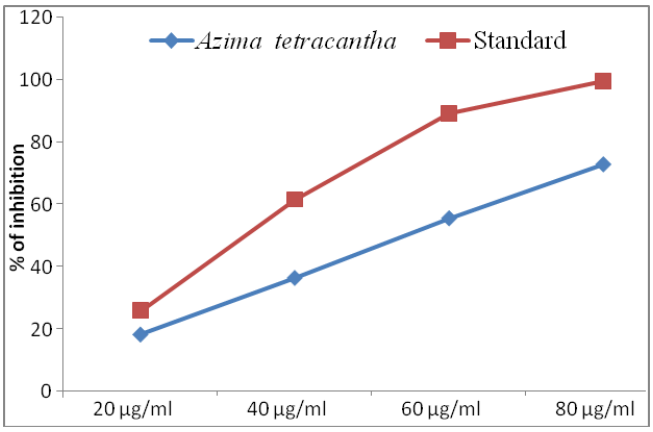

Figure 1: DPPH radical scavenging activity of *Azima tetracantha*

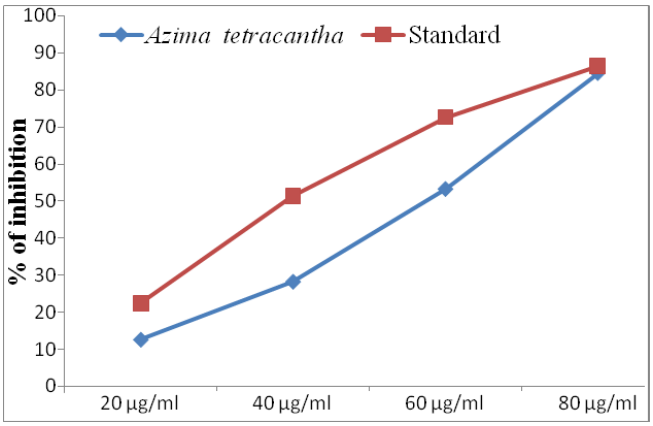

Figure 2: Total Antioxidant Assay of *Azima tetracantha*

Superoxide anion radical scavenging activity

Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals, which are very harmful to the cellular components in a biological system (Korycka-Dahl & Richardson, 1978). The superoxide anion radical scavenging activities of the extract from *Azima tetracantha* assayed by the PMS-NADH system were shown in Fig 3 and Table 2. The superoxide scavenging activity of *Azima tetracantha* was increased markedly with the increase of concentrations. The half inhibition concentration (IC_{50}) of *Azima tetracantha* was 55.93µg ml^{-1} and ascorbic acid were 31.62µg ml^{-1}**.** These results suggested that *Azima tetracantha* had notably superior superoxide radical scavenging effects.

The ferrous ion chelating activity

Ferrozine can make complexes with ferrous ions. In the presence of chelating agents, complex (red colored) formation is interrupted and as a result, the red color of the complex is decreased. Thus, the chelating effect of the coexisting chelator can be determined by measuring the rate of color reduction. The formation of the ferrozine– Fe^{2+} complex is interrupted in the presence of aqueous extract of *Azima tetracantha* , indicating that have chelating activity with an IC_{50} of 50.83µg ml^{-1} and ascorbic acid was 30.96µg ml^{-1} (Fig. 4 and Table 2). Ferrous ion can initiate lipid peroxidation by the Fenton reaction as well as accelerating peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals (Halliwell, 1991). Metal chelating activity can contribute in reducing the concentration of the catalyzing transition metal in lipid peroxidation. Furthermore, chelating agents that form bonds with a metal are effective as secondary antioxidants because they reduce the redox potential and thereby stabilize the oxidized form of the metal ion (Gordon, 1990). Thus, *Azima tetracantha* demonstrate a marked capacity for ion binding, suggesting their ability as a peroxidation protector that relates to the ion binding capacity.

Table 2: *In vitro* antioxidant study of *Azima tetracantha*

Concentrations (µg/ml)	Superoxide Anion	Superoxide Anion (Standard)	Fe^{2+} Chelating Agent	Fe^{2+} Chelating Agent (Standard)
20	14.28±1.00	31.25 ± 2.50	15.48±1.08	35.23 ± 2.81
40	28.57±2.00	64.23 ± 5.13	30.86±2.14	65.21 ± 5.28
60	57.14±4.00	89.54 ± 7.16	65.46±4.58	78.51± 6.28
80	75.00±5.25	98.51 ± 7.88	80.76±5.92	98.65 ± 7.89
IC-50	55.93	31.62	50.83	30.96

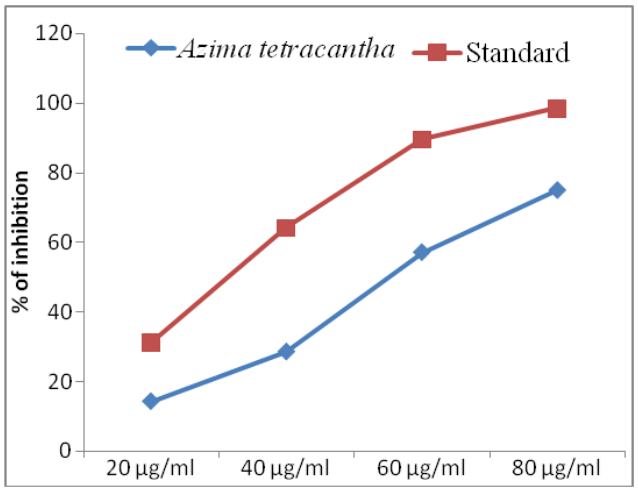

Figure 3: Superoxide radical scavenging activity of *Azima tetracantha*

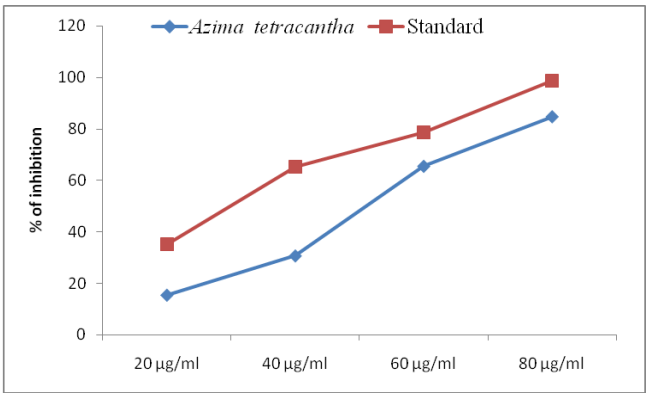

Figure 4: Iron chelating activity of *Azima tetracantha*

Reducing power activity

For the measurements of the reducing ability, the Fe^{3+}–Fe^{2+} transformation was investigated in the presence of *Azima tetracantha* . The reducing capacity of a compound may serve as a significant indicator for its potential antioxidant activity. However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Diplock, 1997; Yildirim *et al.*, 2000). Fig. 5 and

table 3 depicts the reductive effect of *Azima tetracantha* . Similar to the antioxidant activity, the reducing power of *Azima tetracantha* increased with increasing dosage. All the doses showed significantly higher activities than the control indicating that *Azima tetracantha* consist of hydrophilic polyphenolic compounds that cause the greater reducing power.

Table 3: Reducing power activity of *Azima tetracantha*

Concentrations (µg/ml)	*Reducing Power Assay*	*Reducing Power Assay (Standard)*
20	0.24 ±0.017	0.41± 0.03
40	0.44 ±0.031	0.7 ± 0.05
60	0.68 ±0.048	0.89± 0.07
80	0.82 ±0.057	0.98 ± 0.08

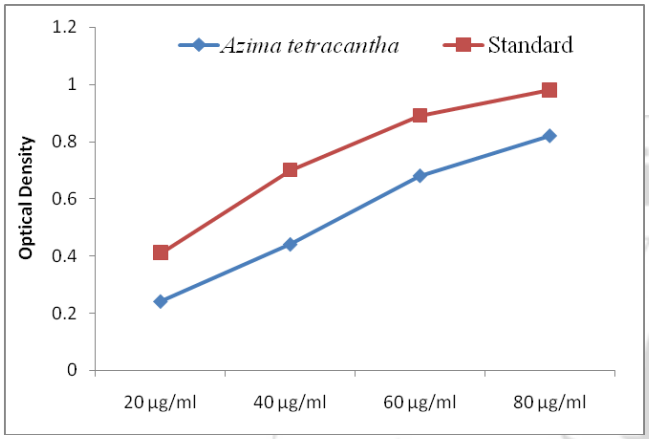

Figure 5: Reducing power activity of *Azima tetracantha*

4. Conclusion

On the basis of the results of this study, it clearly indicates that *Azima tetracantha* leaves had powerful *in vitro* antioxidant capacity against various antioxidant systems as DPPH, superoxide anion scavenging and metal chelator. From our results, the antioxidant activity of *Azima tetracantha* leaves was concentration dependent. The extracts could exhibit antioxidant properties approximately comparable to commercial synthetic antioxidants as ascorbic acid. From the above assays, the possible mechanism of antioxidant activity of *Azima tetracantha* leaves includes reductive ability, metal chelator, hydrogen donating ability and scavengers of superoxide and free radicals.

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