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A Study of the Phytochemical Constituents and the Antioxidant Effects of the Leaf and Flower of *Ixora coccinea* L.

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Abstract
The methanol extracts of the leaf and flowers of *Ixora coccinea* were analyzed for their phytochemical content following standard methods. The reducing power method was used. Different concentrations of *I. coccinea* extract (100µg – 500µg) in 1 ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferric cyanide [K3Fe(CN)6] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3,000 rpm for 10 min. The phytochemical analysis of the leaf extract revealed the presence of alkaloids, flavonoids, tannins, saponins. Methanol extracts and Gallic acid showed antioxidant activity at all concentrations.

Key words: *Ixora coccinea*, antioxidants, Methanol, Plant extracts, Gallic acid.

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Introduction

Ixora coccinea belongs to the family *Rubiacae*, is a common flowering shrub native to Asia including Bangladesh, Southern India, and Sri Lanka (Ghani, 2003). It is cultivated throughout India as an ornamental plant (Varier, 2010). *Ixora coccinea*, commonly known as the flame of the woods, flame of the forest, jungle flame, burning love, scarlet ixora, jungle of Geranium, and red ixora, is one such evergreen shrub.

The word "***Ixora***" is a Portuguese version of Iswari, name of Goddess Parvati to which the flowers of *I. coccinea* are offered, while the word "coccinea" is a Latin word meaning Scarlet coloured (Patil, 2007). It bears flowers which are numerous having bright scarlet colour in dense, senssile corymbiform cymes. The fruits, when fully ripe, are used as a dietary source. *I. coccinea* is a dense, multi-branched evergreen shrub, commonly 4–6 ft (1.2–2 m) in height, but capable of reaching up to 12 ft (3.6 m) high. It has a rounded form, with a spread that may exceed its height. The glossy, leathery, oblong leaves are about 4 in (10 cm) long, with entire margins, and are carried in opposite pairs or whorled on the stems. Small tubular, scarlet flowers in dense rounded clusters 2-5 in (5–13 cm) across are produced almost all year long (Baliga and Kurian, 2012).

A wide variety of oxygen free radicals and other reactive species are formed in the human body and food system (Cui *et. al.*, 2004). It has been reported by Raghuveer and Tandon (2009), that free radicals play an important role in affecting human health by

causing several chronic diseases, such as cancer, diabetes, aging, atherosclerosis, hypertension, heart attack and other degenerative diseases. The action of free radicals however, is blocked by antioxidant substances present in plant extracts which scavenge the free radicals and detoxify the organism. Nowadays, there is a noticeable interest in antioxidants, especially in those which can prevent the presumed deleterious effects of free radicals in the human body, and to prevent the deterioration of fats and other constituents of foodstuffs. In both cases, there is a preference for antioxidants from natural sources rather than from synthetic sources (Molyneux, 2004). The use of plant extracts as natural antioxidants has received increased interest due to the concerns on negative health effects developed by the use of synthetic antioxidants (Abramovic and Abram, 2006; Kowalski, 2007; Azizkhani and Zandi, 2009). Previous studies reported that butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) which are synthetic antioxidants accumulate in the body and result in liver damage and carcinogenesis (Jiangning *et al.*, 2005).

Materials and Methods

Source and preparation of plant materials

The plant parts were collected from the front of the girls hostel of the Gombe State University campus (latitude 11.07º N, longitude 7.73º E and altitude 613meters) and were washed under tap water. The leaves and flowers were shade dried for ten days and then powered to get a coarse powder and were stored in air tight containers. 50g of each part powder was extracted by maceration in 300mL of methanol for 4 days with frequent agitation. The mixture was filtered through clean muslin cloth followed by double filtration with Whatman No. 1 filter paper. The solvent was completely removed by a rotatory evaporator.

Phytochemical screening

The obtained crude extract of flowers and leaves were used for phytochemical screening following the methodology of Trease and Evans (1989).

Reducing power assay

The reducing power of *I. coccinea* was determined according to the method previously described (Oyaizu, 1986). Different concentrations of *I. coccinea* extract (100µg – 500µg) in 1 ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [K3Fe(CN)6] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3,000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml. 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Gallic acid was used as a reference standard. Phosphate buffer (pH 6.6) was used as blank solution. All tests were performed in triplicate and mean values were calculated.

Results and Discussion

The phytochemical analysis of the leaf extract reveals the presence of alkaloids, Flavonoids, tannins, saponin. It has been reported that the leaf of *Ixora coccinea* that was not extracted using any solvent (dry powered sample was used) revealed the presence of flavonoids, tannins, steroids and alkaloids (Damles and Sharon, 2017). It has been reported by Poornima and Suresh (2013) that the aqueous and hydroethanolic extract of the leaf revealed the presence of flavonoids, saponin and tannins while the ethanol extract revealed the presence of flavonoids and saponin with tannins being absent. The phytochemical analysis of the flower extract reveals the presence of alkaloids, flavonoids, tannins, saponins and steroids. The presence of these phytochemical components present in flower extract agrees with the report by Moni *et al* (2008). It has been reported that the ethanol and methanol extract of the flower revealed the presence of alkaloids, flavonoids and

tannins but all these phytoconstituents were found absent in the aqueous extract (Raju *et a*l., 2015). Sumathy *et al.* (2012) revealed that ethyl acetate and methanol flower extract showed the presence of flavonoids, tannins, alkaloids and saponin while petroleum ether flower extract showed the presence of only flavonoids. Flavonoids have been reported to be very good antioxidant agents (Cui *et al.*, 2004). One of the mechanisms of action of flavonoids is through the chelation of transitional metal elements. It enables them to chelate or bind to metal ions in the human body to prevent them from being accessible for oxidation (Malesev and Kunti, 2007). Flavonoids can also act as an intercellular antioxidant through inhibition of free radical generating enzymes such as xanthine oxidase, lipoxygenase, protein kinase C, cyclooxygenase, microsomal monooxygenase, mitochondrial succinoxidase and NADPH oxidase (Malesev and Kunti, 2007).

It has been reported by Majorie (1999) that different solvents had different extraction capabilities and spectrum of solubility for phyto-constituents which may account for the differences observed in the presence of phyto-constituents.

Reducing power assay is used for the measurement of antioxidant activity in the extract. The reducing agents in the extract reacts with the potassium ferricyanide [Fe^{3+}] to form potassium ferrocyanide [fe^{2+}], which then reacts with ferric chloride to form ferrous complex that has an absorption maximum at 700nm (Fejes *et al*., 2000). Increasing absorbance at 700nm indicates an increase in reductive ability (Nabavi *et al*., 2008).The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Ravi and Sandhya, 2012). From figure 1, it was found that the absorbance of the extract and Gallic acid increased with increase in concentrations. It has been reported that flower and leaf and stem extract of *Ixora cocinea* increased with increase in concentration using Diphenyl-1-picryhydrazyl (DPPH) free radical scavenging activity (Moni *et a*l., 2008; Ravi *et al*., 2013; Torey *et a*l., 2010) Reducing power capabilities of extracts was found to be closer to Gallic acid. It was also observed that the absorbance of the extract and Gallic acid increased with increase in concentrations. Reducing power capabilities of flower extract was found to be closer to Gallic acid than reducing power capabilities of leaf extract. This means that the reducing ability of flower extract is higher than the reducing ability of leaf extract. Torey, *et al.* (2010) also showed the methanol extracts of flowers were more effective than the leaf and stem in scavenging DPPH and this may be due to the higher concentration of polyphenols in the flower extract.

Table 1.1: Phytochemical screening of *Ixora coccinea*

Extract	Leaf	Flower
Alkaloids	+	+
Steroids	-	+
Flavonoids	+	+
Saponin	+	+
Tannins	+	+

+ = present, - = absent

Table 2: Reducing power assay of leaf extract

Concentration (µg/ml)	Absorption ± SEM
100	0.1065 ± 0.0081
200	0.1907 ± 0.0042
300	0.2117 ± 0.0051
400	0.3615 ± 0.0040
500	0.3849 ± 0.0013

Values are ±SEM, n=3, µg/ml = microgram/milimitre, SEM = Standard Error of Mean

Table 3: Reducing power assay of flower extract

Concentration (µg/ml)	Absorbance ± SEM
100	0.0642 ± 0.0005
200	0.0716 ± 0.0003
300	0.0867 ± 0.0004
400	0.0941 ± 0.0003
500	0.1051 ± 0.0021

Values are mean ±SEM, n=3

Table 4: Reducing power assay of gallic acid

Concentration (µg/ml)	Absorbance ± SEM
100	0.0414 ± 0.0010
200	0.0582 ± 0.0009
300	0.0668 ± 0.0008
400	0.0753 ± 0.0005
500	0.0836 ± 0.0003

Values are mean ±SEM, n=3

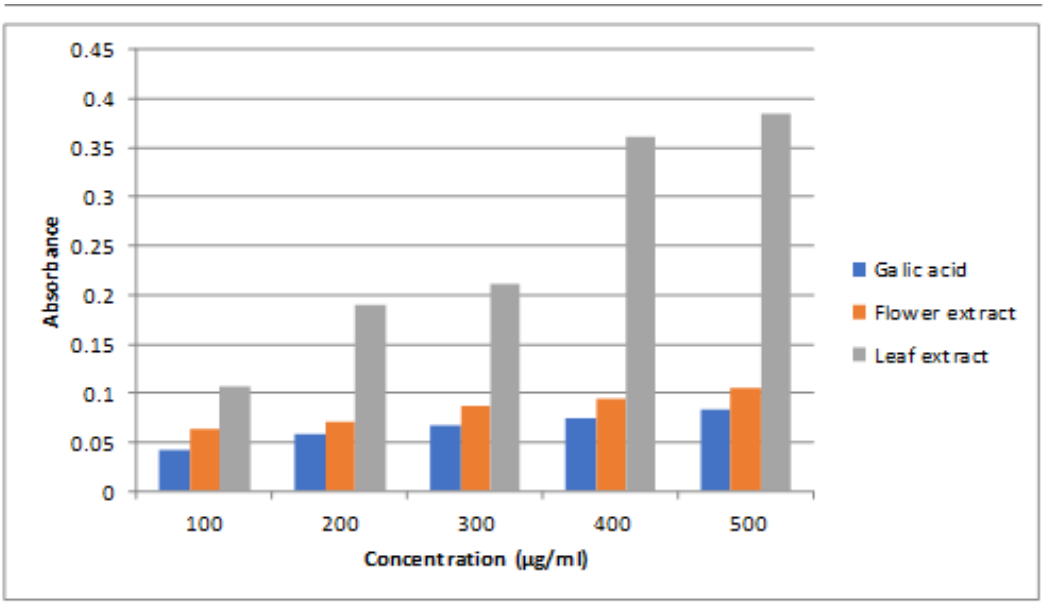

Figure 1: Absorbance of Gallic acid and extracts at different concentrations

Conclusion

The methanol extract of *I. coccinea* possesses the antioxidant substances and appears to be an alternative to synthetic antioxidants. The plant extract also contains different phytochemical components which are responsible for varied pharmacological and medicinal property.

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