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Research Article

EXPLORING THE POTENTIALITIES OF *IXORA LUTEA* (HUTCH) AS A NATURAL DYE AND AN ANTIMICROBIAL AGENT

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ABSTRACT

Many local and exotic plants are of pharmacological importance and hence these are reservoirs of bioactive compounds. The flowers of many plants can serve the purpose of a dye. The present study was undertaken to identify the potentialities of the extracts *of Ixora lutea* (Rubiaceae) as an effective antimicrobial agent and a natural dye. The preliminary phytochemical analysis of the stem and leaf extracts in various solvents (polar and non-polar) revealed the presence of alkaloids, carbohydrates, phenols, tannins, saponins, reducing sugar, triterpenoids and steroids. Both stem and leaf extracts showed great antibacterial and antifungal properties. The noted feature was that the leaf extracts of *Ixora lutea* were proven to have the major antimicrobial activity. The study also deals with the extraction of natural dye from the flower and their application on textiles. The extracted dye along with the mordants gave varying shades of colours on the fabric.

Keywords: *Ixora lutea*, phytochemistry, Antimicrobial, natural dyes and Agar Well Diffusion Method.

1. INTRODUCTION

The use of herbs is becoming popular in the modern times. The herbal products today signify safety in contrast to the synthetic products which are regarded as dangerous to human health and lifestyle (Joy *et al.* 2001). Although herbs have been used as flavoring agents since years, the emergence of synthetics had surpassed their value in the modern industry (Joy *et al.,*2001). But due to the safety issues and health concerns, people are now returning to the use of natural products. As per the studies, now about three-quarters of the world population depend on plants and plant products for health care and various industrial purposes.(Joy *et al.*2001,). Of the 2,50,000 higher plant species in the world, about 80,000 species or more are of medicinal value. India, one of the mega diverse nations has over 45000 different plant species. Of these, about 15000-20000 are probably medicinal. In spite of having a huge diversity of medicinal plants, only 7000-7500 species are exploited by the traditional communities for medicinal purposes. In India, plant extracts have been used as drugs in medicine systems like Unani and Ayurveda (Joy *et al.,*2001). The drugs are derived from the different plant parts like leaves, stem, bark, root, flower, seed, etc. Medicinal plants now occupy a great position in the modern medicine systems for the cure for many human diseases as they contain components of therapeutic value (Adegoke *et al.,*2009). Many plants are sources of active raw materials like a variety of secondary metabolites, which are considered to be the remedies for major ailments.(Audu *et al.,*2007).

Recently, the use of natural dyes has been of great importance because of the toxic and allergic reactions associated with synthetic dyes (Kamel *et al.,*2005). Many researches reveal that synthetic dyes may release harmful chemicals that are allergic, carcinogenic and unsafe to human health.

Since the natural dyes are environment-friendly, its use would prove to be a best substitute to synthetic dyes. Nature has provided us with hundreds of dye-producing plants around us. The proper and wise utilization of these plants would be beneficial.

The genus *Ixora* Linn. with about 400 species are majorly distributed in the tropical and

subtropical regions of the world. In India, this genus has about 43 species (Husain and Paul., 1988).

Ixora are grown in Indian homes due to their brightly coloured flowers and green foliage (Bor & Raizada., 1982, Hussain & Paul., 1989). Their flowers are used for offerings to the deities (Bor & Raizada., 1982). Various species of the genus are used in the traditional medicine systems of India (Udayan & Indira., 2009). *Ixora lutea* is one of the varieties of common *Ixora* plant.

TAXONOMIC HIERARCHY

KINGDOM	PLANTAE
SUBKINGDOM	VIRIDAEPLANTA
DIVISION	TRACHEOPHYTA
INFRADIVISION	ANGIOSPERMAE
CLASS	MAGNOLIOPSIDA
ORDER	GENTIANALES
FAMILY	RUBIACEAE
SUB-FAMILY	IXOROIDEAE
TRIBE	IXOREAE
GENUS	*Ixora*
SPECIES	*Lutea*

Many studies had been conducted to identify the pharmacological properties of various species of Ixora. According to the various studies conducted the genus *Ixora* possesses various types of secondary metabolites mainly triterpenoids, tannins, saponins, carbohydrate, fatty acids, flavonoids and sterols (Dontha *et al.,* 2015).

According to the study conducted by Latha *et al.,*, the methanolic extracts of different parts of *Ixora* species showed a broad-spectrum of antibacterial and antiyeast activities. There wasn't remarkable variation between the activities of different *Ixora* species observed in this study.

But other studies conducted showed that not only the methanolic extracts, but all the 50% ethanolic extracts(Latha *et al.,*2012) and ether extracts(Annapurna *et al.,*2003) isolated possess antimicrobial activities. Moreover, the ether extract was shown to be more effective that the methanolic extract (Annapurna *et al., 2003*).

In a recent work performed, antimicrobial activity was least observed in the leaves of I. *chinensis, I. lutea, I. coccinea* while the leaves of *I. parviflora* showed significant antimicrobial activity. This is in contrast to the previous works, but then they suggest that this variation may be due to differenced in the concentrations of the various extracts used for the work (Akter *et al.,*2015).

Studies conducted also reveal that the plants in the genus Ixora have high antioxidant activity (Moni *et al.,*2008)

In a work conducted by Monali *et al.,*2013, the Methanolic extract of Ixora coccinea (Linn.) flower extracted more pigments and exhibits dark shades on cotton fabric as compared to aqueous extracts.

But the works on *Ixora lutea* are much less when compared to the other species of Ixora. The present study was aimed at identifying the phytochemical constituents and antimicrobial properties of the stem and leaf extracts of *Ixora lutea.*

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The fresh healthy plant material of *Ixora lutea* was collected from various domestic sites of Kollam and authenticated by a botanist. The leaves and stem were dried in shade for a week. The dried leaves and stem were ground into a fine powder.

2.2 Preparation of Solvent Extracts

The fine powder of stem and leaves were separately extracted with various solvents like petroleum ether, chloroform, acetone, methanol, Distilled Water. The solvents were chosen in accordance with the polarity gradient and the method opted for extraction was the simple extraction method.

2.3 Preliminary phytochemical screening

The phytochemical screening were performed on these various solvent extracts according to the standard procedures described by Sofowora(1993) ,Trease & Evans (1989) and Horborne(1937) . The test for proteins, carbohydrates, phenols, tannins, saponins, flavonoids, alkaloids, reducing sugar, coumarins , steroids and triterpenes were conducted.

2.4 Antimicrobial screening

The Agar Well Diffusion Method was used to test antimicrobial activity of the leaf and stem extracts against five bacteria (*Bacillus cereus, Staphylococcus aureus, , Escherichia coli, Salmonella. typhi, and Zymomonas mobilis*) and five fungi (*Aspergillus niger, A.fumigatus, A.flavus, A,flaviceps and Rhizopus oryzae*,).

The bacteria were allowed to grow on Muller-Hinton Agar (MHA) plates. Muller-Hinton agar plates were prepared and the bacterial cultures were swabbed into the surface of the agar plates and wells were punctured on the plates using sterile gel puncture (5mm). Using micropipette, 50 ml of the sample extract was poured on each well on all plates. The plates were incubated at 30-37^{0}C for 24 hours and examined for zones of inhibition.

The fungi (selected test organisms) were grown on Potato Dextrose Agar (PDA) plates. The fungal cultures were swabbed onto the surface of the PDA plates and wells were

punctured using sterile gel puncture (5mm). Using micropipette, 50 ml of the sample extract was poured on each well on all plates. The plates were incubated at 30-37^{0}C for 72 hours and examined for zones of inhibition. Those extracts having antimicrobial activity would have inhibited the growth of the microorganisms around it and a clear zone of inhibition would be visualized surrounding the extracts. The antimicrobial activity of the extracts was determined by measuring the diameter of zone of inhibition expressed in mm.

2.5 Extraction of Natural Dye

2.5.1 Preparation of Floral Extract:

10 g of Fresh floral petals of *Ixora lutea* (Hutch) and boiled in distilled water at 100ºC for 30 minutes for the floral petals to discharge color in distilled water. It was observed that, color of the dye extract was dark red. The solution was filtered for immediate use.

2.5.2 Scouring of cotton

Cotton fabric was washed in a 100 mL solution of 10% Sodium Hydroxide. The scoured material was thoroughly washed with tap water and dried at room temperature.

2.5.3 Dyeing

The cotton fabric was dyed with dye-mordant mixture extract by keeping Mordant: Dye ratio as 1:2. The mordants used were Ferrous Sulphate and Copper Sulphate. The mixture was kept in a boiling water bath for 2 hours. After dyeing, the dyed material was washed with cold water and dried at room temperature. After this it was washed with non- ionic detergent and dried under shade.

3. RESULTS

3.1 Phytochemistry

The preliminary phytochemical analysis has revealed the presence of the following constituents:

- ✓ Proteins
- ✓ Carbohydrates
- ✓ Phenols and Tannins
- ✓ Saponins
- ✓ Alkaloids
- ✓ Reducing Sugar
- ✓ Steroids
- ✓ Triterpenes

Table 1: Phytochemical constituents present in the various solvent extracts of the stem of *I.lutea*

Sl.No	Solvent Extracts	Protein	Carbohydrate	Saponins	Phenols &Tannins	Alkaloids	Reducing Sugar	Flavonoids	Steroids& Triterpenes
1	Petroleum Ether	-	-	+	-	-	-	-	-
2	Acetone	-	+	-	+	+	-	-	-
3	Methanol	-	+	-	+	+	-	-	-
4	Chloroform	-	-	-	-	+	+	-	+
5	Distilled Water	-	+	-	+	-	-	-	-

Table 2: Phytochemical constituents present in the various solvent extracts of the leaf of *I.lutea*

Sl.No	Solvent Extracts	Protein	Carbohydrate	Saponins	Phenols &Tannins	Alkaloids	Reducing Sugar	Flavonoids	Steroids& Triterpenes
1	Petroleum Ether	-	-	-	-	-	-	-	-
2	Acetone	-	+	-	+	+	-	-	-
3	Methanol	-	+	+	+	+	-	-	-
4	Chloroform	+	-	-	-	+	+	-	+
5	Distilled Water	-	-	+	+	+	-	-	-

3.2 Antimicrobial Property

Both the leaf and stem extracts of *Ixora lutea* have considerable antibacterial activity. All the solvents extracts of both stem and leaf had significant effect against the five chosen bacteria of which, the methanolic, acetonic and chloroform extracts were found to be superior. The stem and leaf extracts are proved to have a huge effect against *Zymomonas mobilis.*

Table 3: Antibacterial activity of the solvents against the five chosen bacteria (CONTROL)

		DIAMETER OF INHIBITION ZONE(mm)				
Sl.No	Bacteria	Petroleum Ether	Acetone	Methanol	Chloroform	Distilled Water
1	*B.cereus*	**8**	**7**	**10**	**9**	**8**
2	*Z.mobilis*	-	-	-	-	-
3	*E.coli*	**4**	**12**	**11**	**13**	**3**
4	*S.typhi*	**1**	**10**	**10**	**16**	**10**
5	*S.aureus*	**3**	**12**	**18**	**5**	**1**

Table 4: Antibacterial activity of the solvent extracts of the stem of *Ixora lutea* against the five chosen bacteria

		DIAMETER OF INHIBITION ZONE(mm)				
Sl.No	Bacteria	Petroleum Ether extract	Acetonic Extract	Methanolic Extract	Chloroform Extract	Distilled Water Extract
1	*B.cereus*	**12**	**16**	**14**	**30**	**12**
2	*Z.mobilis*	**15**	**17**	**20**	**10**	**6**
3	*E.coli*	**8**	**15**	**20**	**14**	**5**
4	*S.typhi*	**4**	**12**	**17**	**32**	**10**
5	*S.aureus*	**3**	**34**	**24**	**10**	**2**

Table 5: Antibacterial activity of the solvent extracts of the leaves of *Ixora lutea* against the five chosen bacteria

		DIAMETER OF INHIBITION ZONE(mm)				
Sl.No	Bacteria	Petroleum Ether extract	Acetonic Extract	Methanolic Extract	Chloroform Extract	Distilled Water Extract
1	*B.cereus*	**9**	**7**	**13**	**11**	**8**
2	*Z.mobilis*	**19**	**20**	**25**	**12**	**15**
3	*E.coli*	**11**	**18**	**16**	**21**	**17**
4	*S.typhi*	**13**	**13**	**16**	**25**	**15**
5	*S.aureus*	**10**	**17**	**21**	**19**	**12**

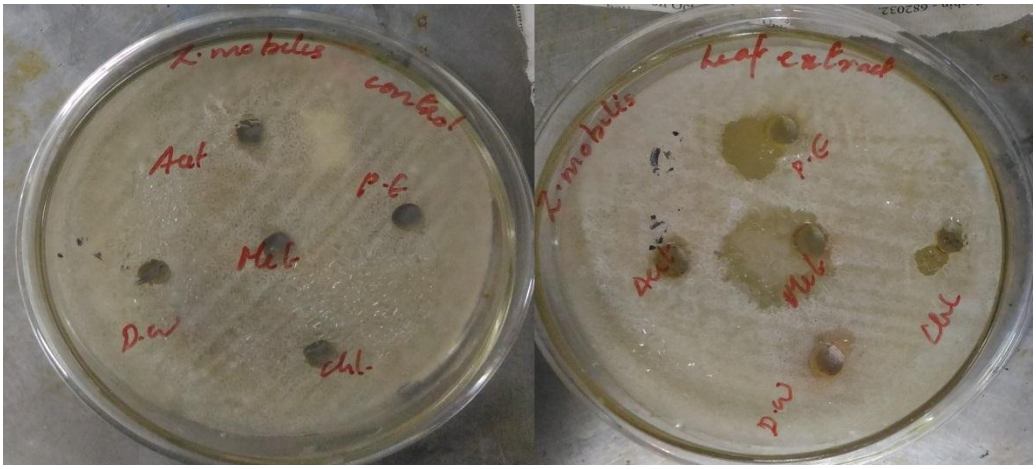

Fig. 1: Activity of the Leaf extracts of *Ixora lutea* against *Zymomonas mobilis*

Both the stem and leaf extracts exhibit antifungal properties, but leaf extracts seems to efficient against the five chosen fungi. However, the antifungal activity of Ixora lutea is considerably less when compared to the antibacterial property.

Table 6: Antifungal activity of the solvents against the five chosen fungi (CONTROL)

		DIAMETER OF INHIBITION ZONE(mm)				
Sl.No	Fungus	Petroleum Ether	Acetone	Methanol	Chloroform	Distilled Water
1	*A.flaviceps*	-	-	-	-	-
2	*R.oryzae*	-	-	-	-	-
3	*A.flavus*	-	-	**1**	**1**	-
4	*A.fumigatus*	-	-	-	-	-
5	*A.niger*	-	**2**	**2**	-	-

Table 7: Antifungal activity of the solvent extracts of the stem of *Ixora lutea* against the five chosen fungi

		DIAMETER OF INHIBITION ZONE(mm)				
Sl.No	Fungus	Petroleum Ether extract	Acetonic Extract	Methanolic Extract	Chloroform Extract	Distilled Water Extract
1	*A.flaviceps*	**17**	**7**	**8**	**15**	**5**
2	*R.oryzae*	-	-	-		-
3	*A.flavus*	**2**	**10**	**5**	**2**	**1**
4	*A.fumigatus*	-	-	**7**	**8**	-
5	*A.niger*	**1**	**2**	**2**	**12**	**1**

Table 8: Antifungal activity of the solvent extracts of the leaves of *Ixora lutea* against the five chosen fungi

		DIAMETER OF INHIBITION ZONE(mm)				
Sl.No	Fungus	Petroleum Ether extract	Acetonic Extract	Methanolic Extract	Chloroform Extract	Distilled Water Extract
1	*A.flaviceps*	12	9	10	17	6
2	*R.oryzae*	-	2	3	1	1
3	*A.flavus*	4	12	4	2	1
4	*A.fumigatus*	1	2	7	9	1
5	*A.niger*	5	7	9	12	2

3.3 Dye- Extraction

A reddish dye was obtained from the flowers of *I.lutea* with the mentioned method of extraction. Effect of the dye colour with the mordants and the effect of dye without mordant are presented in the tables below.

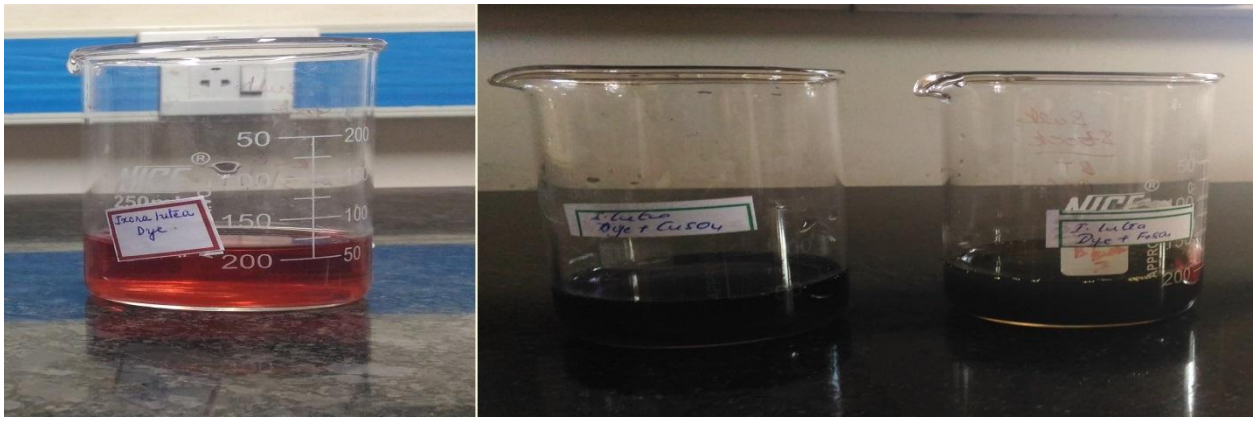

Fig. 2: Images of the Crude dye and the dye mixed with mordants

Fig. 3: Colours obtained in the cotton fabric. A: Cotton fabric dipped in *I.lutea* dye without the mordant; B- Cotton fabric dipped in *I.lutea* dye with $FeSO_4$ s the mordant; C- Cotton fabric dipped in *I.lutea* dye with $CuSO_4$ as the mordant

The cotton fabric dipped in the Dye-$CuSO_4$ mordant mixture showed the Colour Olive Gleam (No: M209 on Asian Paints Colour Catalogue), while the cotton fabric immersed in the Dye+$FeSO_4$ mixture showed the Mustard Colour (7901 on Asian Paaints Colour catalogue)

4. DISCUSSION

The antimicrobial activity and the phytochemical screening of the stem and leaf extracts of *Ixora lutea* along with the extraction of dye from its flowers was carried out and the results are tabulated.The phytochemical constituents present in the stem and leaf extracts of *Ixora lutea* were proteins, carbohydrates, reducing sugar, phenols and tannins, alkaloids, saponins, steroids and triterpenes. Previous findings suggests the presence of tannin, saponins, phenols and other phytoconstituents in the genus *Ixora* and hence it can be correlated with the present study.(Dontha *et al., 2015*)

The various extracts were tested against selected bacterial strains and five fungal strains. The results reveal that *Ixora lutea* possess strong antimicrobial activity.Among the selected bacterial strains, both the stem and leaf extracts were very much efficient in inhibiting the growth of *Zymomonas mobilis*. Among the solvent extracts, the methanolic extracts and chloroform extracts had strongly inhibited the growth of the bacterial colonies.Now, the antifungal property *of Ixora lutea* against five fungal strains was also studied. In the present work, the stem extracts had less antifungal activity. Moreover, the stem extracts did not inhibit the growth of *R.oryzae*. But the antifungal activity of the leaf extracts was substantially high when compared to that of the stem extracts. Among the solvent extracts of both stem and leaf, the chloroform extracts had the most antifungal activity followed by the methanolic extracts.

Previous works reports on the antimicrobial activity of *Ixora lutea* can be compared with the present study as there are some differences and similarities in the result. According to the results reported by Latha *et al.,*, the methanolic extracts of different parts of *Ixora* species showed a broad-spectrum of antibacterial and antiyeast activities (Latha *et al., 1995*). This is in accordance with our present work as the methanolic extracts of stem and leaf *of Ixora lutea* were found to be a strong antibacterial and antifungal agent. Another study conducted to analyze the antimicrobial activity of *I. chinensis, I. lutea, I. coccinea and I. parviflora* almost no antimicrobial activity was reported in *I. lutea*.(Akter *et al., 2015*) This is in contrast to the present study, but we assume that this difference might be due to the concentration of the extracts used for the tests.

The work also aimed at the extraction of dye from the flowers of *I.lutea*. A reddish dye was obtained from the aqueous extract of the flowers of *I.lutea*. The natural dyes obtained from the flowers are a type of pigmentary molecules which helps impart colour to materials. Mordants are chemicals which help retain and fix the colour of the dyes. The mordants used in this study gave varying shades in the cotton fabric. Better color strength results are dependent on the metal salt used in the mordant. (Kamel *et al.2009,*). Wash fastness of dye is usually influenced by the rate of diffusion of dye inside the fiber. The dye extracted from Ixora lutea flowers exhibited an excellent wash fastness. Present study indicated the changes of some colours in the dyed samples of cotton fabric with different mordents. The use of copper and ferrous sulphate as mordants gives high resistance to fading. The use of natural dyes is gaining prime importance owing to its biodegradability and quick availability. They are non-toxic, non-allergic to skin, non-carcinogenic and renewable (Kulkarni *et al.,*2011).

The present study indicates that *Ixora lutea* can be used for medicinal purposes owing to its antimicrobial activities. In future studies, many other medicinal aspects of its can be studied, which can pave way for the use of *Ixora lutea* as a major drug for many ailments. Since the present study was successful in extracting a natural dye from its flowers, this flower can be used as a source of dye and can make its use in textile industries.

5.CONCLUSION

Many locally available plants are known to the repositories of various medicinal compounds. The stem and leaf extracts of *Ixora lutea*, a common plant of Indian households, also have a number of medicinally important phytochemical compounds. This accounts for the antimicrobial activity of the plant. Also, the flowers can be utilized as a commercial dye for textiles. The present work thus validates the medicinal and commercial use of the plant, *Ixora lutea*.

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