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Phytochemical composition and bioactivity assessment of *Ixora nigricans* R.Br. ex Wight & Arn. leaf extract

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Abstract

This study aimed to assess the phytochemicals and bioactivities of the methanolic leaf extract to verify the traditional therapeutic uses of *Ixora nigricans* R.Br. ex Wight & Arn. The shrub has historical record of use as Ayurveda and traditional medicine in Bangladesh to treat diverse illnesses. The study examined its oxidation-inhibiting, inflammation-reducing, cell-killing, and bactericidal capabilities. Phytochemical analysis confirmed the presence of alkaloids, coumarins, glycosides, saponins, steroids, tannins, terpenoids, quinines, phenols, and proteins while anthraquinones, flavonoids, and phlobatannins were absent. The leaf extract exhibited significant oxidation inhibiting (IC_{50} = 38.52 µg/mL), inflammation-reducing (IC_{50} = 86.18 µg/mL) and cell-killing (LC_{50} = 30.43 µg/mL) abilities. Additionally, it inhibited the growth of *Escherichia coli* (9.5 mm) and showed moderate bactericidal effects (11mm) at a concentration of 250 µg/mL against *Bacillus subtilis*, *Bacillus cereus*, and *Salmonella typhi*. Overall, these findings corroborate the traditional application of *I. nigricans* and emphasise its capacity to develop novel, natural remedies for diverse health conditions.

Keywords: *Ixora nigricans,* oxidation inhibiting, inflammation-reducing, cell-killing, bactericidal.

Introduction

Ayurveda, a time-honored system of medicine, has been employed for millennia for its possible therapeutic advantages, notably through the utilization of diverse botanicals[1]. A notable shrub *Ixora nigricans* (Rubiaceae), indigenous to Bangladesh[2] is and extensively utilized in traditional medicine and ayurveda to address various health conditions[3]. The plant is referred to by many appellations among indigenous communities in Bangladesh, such as Dikranga, Chuillya among the Chakma and Tripura tribes, and Rongma and Frareko among the Marma[4]. The plant is used by several tribes in Bangladesh for treating various health concerns. For example, the Chakma applies the root extract of *I. nigricans* to alleviate symptoms of diarrhea and ear infections[5]. The Tanchangya formulates pills using leaf paste for dysentery treatment, consumed three times a day. Conversely, the Marma utilizes a root extract as a remedy for unconsciousness in young children, as well as for treating vomiting and bleeding[6]. Multiple investigations on the leaves of *I. nigricans* have revealed strong pharmacological properties, including anti-arthritic, cytotoxic, anti-inflammatory, antioxidant, antiviral, antispasmodic, and central nervous system depressant activities[7-9]. With the growing interest in natural remedies promoting health and well-being, the biological profiling of *I. nigricans* has gained significance. This study aims to evaluate the phytochemical compounds and the antioxidant, anti-inflammatory, cytotoxic, and antibacterial properties of the methanolic extract of *I. nigricans* leaves, thereby confirming its traditional medicinal uses and exploring its potential for developing novel, organic remedies for diverse health conditions.

Materials and Methods

Plant specimen

Leaf specimen of *I. nigricans* was gathered from KEPZ, Chattogram. Afterward, a voucher specimen (49-2019_KEPZ) was deposited at the Herbarium of Chittagong University (HCU), Department of Botany, for future reference.

Processing of plant material

The leaves were cleansed, fragmented, and allowed to dry naturally at the ambient temperature. Coarse powder was prepared from the dried leaves and kept in an air tied jar. The powder then filtered using Whatman No. 1 filter paper to create the methanolic extract, which was subsequently dehydrated under vacuum at a temperature less than 50°C to produce a dark-colored section.

Phytochemical screening

The methanolic extract and crude powder were screened for alkaloids, flavonoids, glycosides, saponins, steroids, tannins, terpenoids, anthraquinones, quinine, phenols, coumarins, phlobatannins, and proteins following standard methods[10-17]. The intensity of precipitation or colour change was graded as '+' (slight), '++' (moderate), '+++' (high), or '–' (absent).

Evaluation of bioactivities

The extract was evaluated for its antioxidant, anti-inflammatory, cytotoxic, and antibacterial activities following standard procedures[18-21].

Results and Discussion

Phytochemical profile

The phytochemical analysis of *I. nigricans* leaves revealed a diverse range of bioactive compounds detected in the leaf extract (**Table 1**), for example alkaloids, coumarins, glycosides, saponins, steroids, tannins, terpenoids, quinones, phenols, and proteins were identified; however, flavonoids, anthraquinones, and phlobatannins were absent.

These findings are consistent with traditional uses of the plant and suggest a robust phytochemical profile that could contribute to its medicinal properties. The absence of flavonoids, anthraquinones, and phlobatannins may indicate specific functional roles of the detected compounds in the plant's therapeutic efficacy. Alkaloids, for instance, are known for their potent biological activities, including anti-inflammatory and cytotoxic effects[22]. Coumarins and glycosides also contribute to antioxidant and antimicrobial properties, which align with the reported traditional uses of the plant[23].

The detected phytochemicals are essential for the development of therapeutic agents. The presence of saponins and steroids is particularly noteworthy, as they are linked to antimicrobial and anti-inflammatory effects[24]. The diversity of compounds present in *I. nigricans* suggests that it has significant potential for developing multifunctional therapeutic agents.

Table 1: Phytochemical profile of *I. nigricans* leaf extract

Alkaloids					Gly.	Sap.	Ste.	Tan.	Ter.	Qui.	Phe.	Cou.	Pro.	Flv.	Ant.	Phl.
D	H	M	W	T												
++	+++	++	+++	+++	+	+	+	+	+	+	+	+	+	-	-	-

Note: D-Dragendroff's reagent, H-Hager's reagent, M-Mayer's reagent, W-Wagner's reagent and T- Tannic acid reagent, Gly.=Glycosides, Sap.=Saponins, Ste.=Steroids, Tan.=Tannins, Ter.=Terpenoids, Qui.=Quinine, Phe.=Phenol, Cou.=Coumarin, Pro.=Protein, Flv.=Flavonoids, Ant.=Anthraquinone, Phl.=Phlobatanins

Antioxidant activity

The antioxidant activity of the *I. nigricans* leaf extract was assessed using the DPPH radical scavenging assay. The extract exhibited an IC_{50} value of 38.52 µg/mL (**Figure 1**), demonstrating substantial antioxidant potential, although slightly less than Ascorbic acid, which had an IC_{50} of 34.31 µg/mL. These findings suggest that the extract had a strong capacity to neutralize free radicals, consistent with its traditional use as an antioxidant.

Antioxidants play a critical role in mitigating oxidative stress and preventing cellular damage caused by free radicals[25]. The antioxidant activity of *I. nigricans* leaf extract, with an IC_{50} value of 38.52 µg/mL, indicates a strong capacity to neutralize free radicals, although slightly less potent compared to ascorbic acid (IC_{50} of 34.31 µg/mL). This activity may be attributed to the phenolic content in the extract, which has been shown to correlate with antioxidant properties[26]. The observed antioxidant activity supports the traditional use of *I. nigricans* for combating oxidative stress-related conditions and reinforces its potential as a natural source of antioxidants.

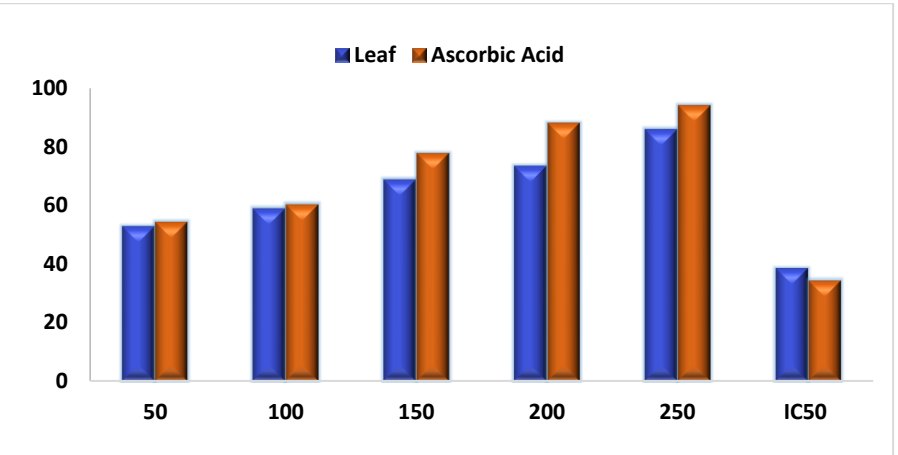

Figure 1 Comparative antioxidant properties of *I. nigricans*

Anti-inflammatory activity

The anti-inflammatory activity of the *I. nigricans* leaf extract was assessed using the protein denaturation inhibition method. The extract showed an IC_{50} value of 86.18 µg/mL, indicating a moderate anti-inflammatory effect compared to acetylsalicylic acid (IC_{50} of 32.84 µg/mL). The percentage inhibition of protein denaturation by the extract was 83% at the concentration of 250 µg/mL, as illustrated in **Figure 2**. These findings suggest that the extract contains compounds that can effectively reduce inflammatory responses, consistent with its traditional use.

The anti-inflammatory activity of *I. nigricans* leaf extract, with an IC_{50} value of 86.18 µg/mL, demonstrates its potential to inhibit inflammation, comparable to the standard acetylsalicylic acid (IC_{50} of 32.84 µg/mL). The ability of the extract to reduce protein denaturation suggests that it may effectively mitigate inflammatory responses[27]. Alkaloids and steroids, which were identified in the extract, are known to possess anti-inflammatory properties and may contribute to these effects[28]. This finding aligns with the traditional use of the plant for treating inflammatory conditions and highlights its potential in developing anti-inflammatory agents[29].

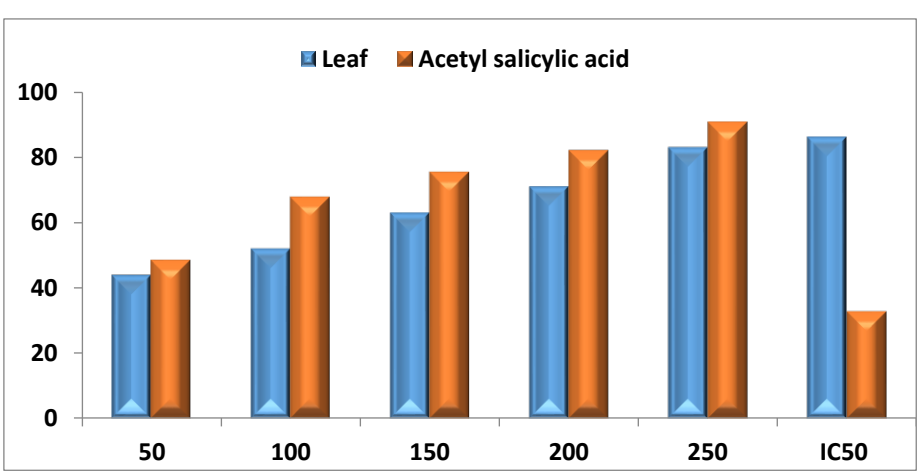

Figure 2 Comparative anti-inflammatory effectiveness of *I. nigricans*

Cytotoxic activity

The cytotoxicity of *I. nigricans* leaf extract was evaluated using the brine shrimp (*Artemia salina*) lethality assay. The extract demonstrated an LC_{50} value of 30.43 µg/mL, indicating significant cytotoxic potential. It is noted that higher concentrations of the extract led to increased mortality in brine shrimp **(Table 2)**. This result suggests that the plant extract may possess compounds that induce cytotoxic effects, possibly through apoptosis or cell cycle arrest mechanisms[30]. Alkaloids and phenolic compounds, identified in the extract, are known for their cytotoxic properties[31-32].

According to the United States National Cancer Institute, the observed LC_{50} value is classified as cytotoxic[33]. This finding supports the need for further investigation into the therapeutic potential of *I. nigricans* in cancer treatment.

Table 2: Cytotoxicity of *I. nigricans* leaf extract

Concentration		Nauplii		Mortality		LC_{50} value (µg/mL)	
						Extract	Standard
µg/mL	LogC	Taken	Dead	Percentage	Probits		
50	1.699	27	18	67	5.44	30.43	0.30
100	2.000	24	17	71	5.55		
150	2.176	27	21	78	5.77		
200	2.301	23	23	100	7.33		
250	2.398	25	24	96	6.75		

Antibacterial activity

The antibacterial activity of the *I. nigricans* leaf extract was determined using the disc diffusion method. The extract exhibited moderate antibacterial activity against several bacterial strains, including *S. typhi*, *B. cereus*, *B. subtilis*, and *E. coli*. The inhibition zones ranged from 7 to 11 mm, with the highest activity observed against *B. cereus* **(**Figure 3**).** This result also confirms the traditional use of the plant for treating bacterial infections and highlights its potential as a natural antimicrobial agent.

The antibacterial activity of *I. nigricans* leaf extract, with inhibitory zones ranging from 7 to 11 mm, demonstrates moderate activity against various bacterial strains, including *S. typhi*, *B. cereus*, *B. subtilis*, and *E. coli*. This activity could be attributed to the presence of saponins, tannins, and terpenoids, which have been reported to exert antimicrobial effects[34]. The findings are aligned with the findings of Rahman *et al.*[35]. Research has demonstrated that tannins hinder the proliferation of bacteria by creating enzyme complexes and causing the precipitation of proline-rich proteins that are attached to the cell membrane[36]. Terpenoids have the ability to degrade microbial membranes, hence inhibiting microbial growth[37]. Conversely, steroids have the ability to cause the release of the contents of liposomes that contain phosphatidyl ethanolamine, thereby restricting the growth of bacteria[38]. The observed antibacterial properties validate the traditional use of *I. nigricans* in treating bacterial infections and suggest its potential as a source of natural antimicrobial agents. The comparative effectiveness against different bacterial strains underscores the plant's potential for developing novel antimicrobial therapies.

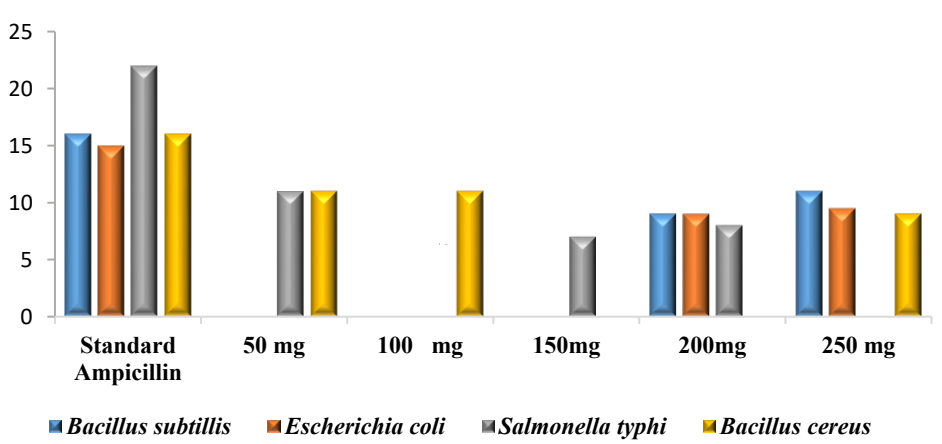

Figure 3 Comparative antibacterial effects of *I. nigricans*.

Conclusion

The comprehensive analysis of *I. nigricans* leaf extract highlights its potential as a source of bioactive compounds with significant antioxidant, anti-inflammatory, cytotoxic, and antibacterial activities. These findings reinforce the traditional uses of the plant and suggest that it holds promise for developing natural therapeutic agents. Future research should focus on isolating and characterizing the specific bioactive compounds responsible for these activities and exploring their mechanisms of action. Additionally, further studies are needed to assess the safety, efficacy, and potential applications of *I. nigricans* in clinical settings.

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