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Ethanollic *Clerodendrum inerme* leaf extract: UV, FTIR spectroscopy and phytochemical screening

Shahin Aziz^{*1}, Md. Morshed Alam², Sharika Farhana²

¹Chemical Research Division, BCSIR Laboratories Dhaka, Bangladesh Council of Scientific and Industrial Research, Dhamondi, Dhaka, Bangladesh

²Department of Applied Chemistry and Chemical Engineering, Islamic University, Kushtia, Bangladesh

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Abstract

One significant medicinal herb is *Clerodendrum inerme*. The plant is referred to locally as "bonjol" in Bangladesh. The current study examines the ethanolic seed extract of this plant using UV and FT-IR spectroscopy as well as phytochemical screening. The plant has anti-inflammatory, anti-cancer, anti-malarial, antidiabetic, and antioxidant qualities. A group of phytochemicals like flavonoids, terpenoids glycosides, phytosterols, etc. are all present in the extract according to phytochemical screening. Carbonyl group (ketone), α,β unsaturated amides, lactams, sulfur compounds, nitro compounds, flavones, fustins, quercetins, Sodium Salts of Quercetin 5' Sulfonic Acid, myricetins, chalcones, flavonoids (anthocyanin type) are detected by UV and Fourier Transform and Infra-Red spectroscopy of the plant's ethanolic leaf extract. The bioactive compounds mentioned above primarily contribute to the plant's therapeutic properties.

***Corresponding Author:** Shahin Aziz ✉ shaziz2408@yahoo.com

Introduction

In Bangladesh, *Clerodendrum inerme* (*C. inerme*) is referred to locally as "bonjol." This plant is a member of the Verbenaceae family. The *C. inerme* tree is a hardy, straggling shrub that grows to a height of 3–4 meters. It is an evergreen mangrove plant with closely spaced, nearly spherical, glossy, deep green leaves. It is a multipurpose plant that may be cultivated as a bonsai or as topiary. The plants typically grow in warm climates like Bangladesh, Malaysia, Vietnam, China, India, Pakistan, and the Philippines (Brickell *et al.*, 1997). Numerous indigenous medical systems and folk remedies have mentioned *C. inerme* (Neeta *et al.*, 2007).

In addition to homeopathy and electropathy, the plant's therapeutic properties have been documented and are used by herbalists, traditional healers, and members of Bangladeshi medical systems, including Ayurveda, Unani, and Siddha. These plants have a significant impact on the nation's population's health (Somasundram *et al.*, 1986). Because *C. inerme* loves the sun, it should be placed in a sunny area. The plant has significant therapeutic potential in many parts. This plant's leaves and roots are used to treat skin conditions and rheumatism (Kothari *et al.*, 2006). Ayurvedic medicine uses several portions of the *C. inerme* plant to treat tumors, beri-beri, venereal infections, rheumatism, and skin conditions. The leaf juice is administered orally to treat tetanus, which is characterized by leg rigidity and muscle soreness. Additionally, rheumatism and skin conditions are treated using the leaves and roots (Manoharan *et al.*, 2006). Cattle with rheumatic discomfort and arthritis are given a fine paste produced from the extract of pounded leaves with pepper asafetida (Kaushik *et al.*, 1999). To treat fever, a leaf is mashed in water and its juice is consumed orally (Harish *et al.*, 2011). For disorders that are susceptible, the roots are recommended. The free sugars are extracted from the dried flowers (Krishnan Marg, 2001). In dogs, its extracts have hypotensive effects. In mice, the methanolic extract of *C. inerme* leaf extracts exhibited antispasmodic properties (Neeta *et al.*, 2007).

According to reports, its leaves are active in the cardiovascular system and have been demonstrated to have antibacterial properties. They also suppress intestinal motility and increase uterine motility in rats. Neolignans, sterols, diterpenes, iridoids, flavonoids, and triterpenes are the plant's primary constituents (Richa *et al.*, 2005); (Heneczowski *et al.*, 2001). Tested on female rats and rabbits, organic extracts of *C. inerme* demonstrated substantial uterine stimulant activity as well as strong antihemolytic activity in human adults (0.02–2.0 mg/mL) with phospholipase inhibition (0.05–1.5 mg/mL) (Somasundram *et al.*, 1986). By altering calcium transport in isolated rat liver inflammation, flavonoid glycosides of *C. inerme* demonstrated a decrease in inflammation. Experiment's outcomes were similar to those of the positive control, indomethacine (Kalyanasundaram *et al.*, 1985). Because *C. inerme* contains a bitter component, reports of its antimalarial properties have been made. Additionally, at 80 and 100 ppm concentrations of petroleum ether and ether extracts, *C. inerme* reduced the growth of *Aedes aegypti*, *Culex quinquefasciatus*, and *Culex pipiens* larvae (Masuda *et al.*, 1999); (Mehedi *et al.*, 1997). Numerous indigenous medical systems have utilized it as an antioxidant drug (Sharma *et al.*, 1979). With an ED₅₀ value of 16 µg/mL, dried, aerial portions of *C. inerme* demonstrated strong antiviral activity against the Hepatitis B virus (George *et al.*, 1949). Antifungal activity against a range of fungal species, including *Microsporum gypseum*, *Mucor mucedo*, *Penicillium digitatum*, *Rhizopus nigricans*, *Trichophyton rubrum*, and *Trichothecium roseum*, was demonstrated by essential oil extracted from the plant's leaves (Rajasekaran *et al.*, 2006). Additionally, alcoholic extracts of *C. inerme*'s leaves and flowers shown antibacterial action against *Staphylococcus aureus* and *Escherichia coli* (Manoharan *et al.*, 2006). According to certain researchers, *C. inerme*'s ethyl acetate extract has antibacterial properties against human infections. Other biological activities, like an antihemolytic action, have been documented for it (Shanmugam *et al.*, 2008). It has been demonstrated that the

plant's leaf extract possesses insecticidal qualities against mosquitoes. Numerous plant-based solvent extracts have been studied for their ability to repel mosquitoes. Investigating the dry powder of leaf material as a source of insecticidal qualities against mosquito larvae was therefore deemed fruitful.

The impact of powdered sun-dried *C. inerme* leaves on *A. aegypti* larvae in their fourth instar (Richa *et al.*, 2005). Indian traditional healers utilize it to treat a number of illnesses, including cancer. It modulates antioxidant defense pathways and lipid peroxidation to achieve its chemopreventive effect (Harwood *et al.*, 2005). 500 mg/kg body weight of *C. inerme*'s aqueous leaf extract taken orally dramatically reduced the development of tumors and histopathological abnormalities. During DMBA-induced oral carcinogenesis, oral administration of *C. inerme* preserved the levels of red blood cell osmotic fragility, cell surface glycol conjugates, blood and tissue lipids, and membrane-bound enzyme activity (Rajasekaran *et al.*, 2006; Bohm, 1998; Caius, 1986).

Numerous phytoconstituents have been identified from different plant sections. 3-Epicaryoptin, which was extracted from the leaves, inhibits the growth of houseflies and mosquitoes and has antifeedant properties. The hexane extract of *C. inerme*'s aerial parts included three novel neoclerodane diterpenoids: inermes A, inermes B, and 14,15-dihydro-15b-methoxy-3-epicaryoptin.

It has also been possible to isolate 14, 15-Dihydro-15-hydroxy-3-epicaryoptin as an epimeric combination (Cooke, 1958).

In order to learn more about the functional groups found in the different secondary metabolites of this significant medicinal plant, the current study aimed to analyze the ethanolic extract of *C. inerme* leaf using UV and FT-IR in conjunction with phytochemical screening. This will help others understand why this plant's leaves are used medicinally (Fig. 1).



Fig. 1. *C. inerme* dry & wet leaves

Materials and methods

C. inerme sample Collection with identification

The taxonomist at the Bangladesh National Herbarium in Dhaka, where a voucher specimen (No. =46305) has been stored, recognized fresh leaves of that were collected at the Dhaka University Campus in May 2018.

Plant materials preparation

The fresh leaves of *C. inerme* that were gathered at the Dhaka University Campus in May 2018 were identified by the taxonomist at the Bangladesh National Herbarium in Dhaka, where a voucher specimen (No. =46305) has been kept.

Solvents and chemicals

In these investigations, chemicals and solvents of analytical or laboratory quality were employed and from BDH, England and some from E Marck, Germany.

C. inerme leaf extract from ethanol: Preparation

During the extraction process, 120 g of powered leaf material is immersed in appropriate solvents with increasing polarity, such as ethanol, and then allowed to sit at room temperature for five days while being shaken and stirred periodically. During this time, the majority of the plant material's extractable chemicals will dissolve in the solvent and be extracted as a solution. A rotary evaporator was then used to dry these extracts, yielding 2.0 g of ethanol extract. In order to identify different plant ingredients, the resulting extract was next put through a preliminary phytochemical screening process using techniques recommended by established methodologies (Durry, 2010; Saraswathi *et al.*, 2012; Dutta, 2000). Using Ultra-Violet and Infra-Red spectral analysis, the functional and chemical group of phytochemicals as well as flavonoids included into the ethanolic extract were identified.

Results and discussion

Phytochemical screening

A group of phytochemicals like Alkaloid, flavonoid, glycoside, phytosterol, terpenoid, phenolic compound, carbohydrate, fixed oil, lipid, protein, tannin, gum, mucilage are present in the *C. inerme* leaf extract from ethanol. Table 1 displays the findings.

Ultra violet spectroscopy

C. inerme ethanolic leaf extract's UV spectrum was measured between 273-292 nm. Due to the aromatic structure of compounds and aldehydes, the UV spectrum exhibits weak absorption bands at 292.28 nm. Flavone and fistein kinds of flavonoids are shown by these weak bands.

Table 1. *C. inerme* Leaf extract from ethanol: Phytochemical profiling

Plants configuration test/ Methods	Observations	Plants configuration test/ methods	Observations
Alkaloid		Carbohydrate	
Reagents			
Mayer's	Present	Glucose	Absent
Wagner's	Present	Fructose	Absent
Hager's	Present	Galactose	Absent
Carbohydrate		Lactose	Present
Molisch's test	Present	Starch	Present
Benedict's reagents	Present	Glycoside	
Fehling solution	Present	Keller Killiani test	Present
Terpenoid	Present	Phytosterol	
Salkowski test	Present	Liebermsnn's test	Present
Fixed oil, Fats		Saponin	
Spot test	Present	Foam test	Absent
Phenolic compounds		Tannins	Present
FeCl ₃ solution	Present	Lead acetate solution	
Proteins	Present	Amino acids	
Xanthoprotic test	Present	Ninhydrine reagents	Present
Biuret test	Present	Flavonoids	
Gums and Mucilages		Con.H ₂ SO ₄ + Mg ribbon	Present
Alcoholic precipitation	Present	Anthraquinones	
Molisch's test	Present	Borntrager's test	Absent

Table 2. *C. inerme* leaf extract from ethanol: UV spectroscopy

Wavelength in nm	Abs.	Chromophoric group	Flavonoids
292.28	0.068	Aldehyde(-CHO)	Flavone & Fistein
289.66	0.070	3° amine, Polyene(β-Carotain,)	Quercetin
288.80	0.071	3° amine, Polyene(β-Carotain,)	Quercetin
287.80	0.070	Amide group (protein).	
285.60	0.066	Amino group (Aniline)	
284.20	0.021	=C=O, CHO	Flavone and Fistein
283.42	0.055	=C=O, CHO	Flavone and Fistein
282.20	0.005	-CHO	Flavone and Fistein
281.84	0.015	-CHO	Flavone and Fistein
281.10	0.115	-CHO	Flavone and Fistein
280.26	0.017	=C=O	Flavone, Fistein
278.20	0.393	=C=O	Flavone , Fistein
277.84	0.402	=C=O	Flavone and Fistein
274.82	0.646	Alkene group (Naphthalene)	-
273.30	0.255	Alkene group (Naphthalene).	-

Quercetin is shown by the absorption band at 289.66 nm and 288.80 nm, which is caused by 3° amine and polyene (β-carotene). The presence of an amide group (protein) is indicated by the distinctive wide band at 287.80 nm. Band at 285.60 nm indicates aniline

presence, by an amino group. At 284.20 and 283.42 nm, the distinctive band is caused by the aldehyde and ketones groups. Flavone and fistein kinds of flavonoids are shown by these distinctive bands. Band at 282.2, 281.84, 281.10 nm shows the presence of

aldehyde groups. The sharp band at 280.26, 278.20, 277.84 nm due to the presence of ketones group. The existence of an alkene group is shown by the band at 274.82 nm and 273.30 m. Three different kinds of flavonoids—flavone, fisetin, and quercetin—are detected by UV spectroscopy (Fig. 2, Table 2).

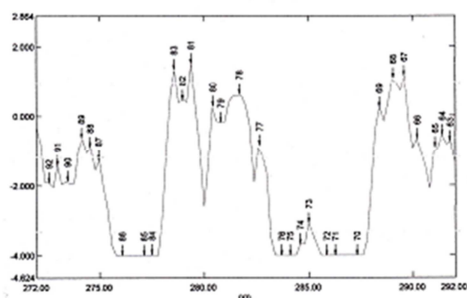


Fig. 2. *C. inerme* leaf extract from ethanol: Ultra violet spectrum

FT-IR spectroscopy

The presence of alkyne, C-H bending vibration amides, and quercetin is indicated by the peak at 736.80 cm⁻¹ in the FT-IR spectrum of an ethanolic extract of *C. inerme* leaves. Gem disubstituted olefinic

group, C-H bending vibrations, and aromatic substitution are the causes of the strong peak at 895.15 cm⁻¹. The existence of quercetin is once again confirmed by this peak. The presence of sulfur compounds, S=O stretching vibrations, thiocarbonyl groups, sulfoxides, and Sodium Salts of Quercetin 5' Sulfonic Acid is indicated by the extremely sharp signal at 1036.04 cm⁻¹. The prominent peak at 1089.91 cm⁻¹ further supports the existence of sulfur compound, thiocarbonyl group, and Sodium Salts of Quercetin 5' Sulfonic Acid.

A sulfur chemical that is highly effective against microorganisms. The existence of C-N Stretch and the functional group aliphatic amine are shown by the peak at 1254.23 cm⁻¹ in the FT-IR spectra. The substance's aromatic character, sulphonamides, gem dimethyl group, nitro compound, and myricetin type of flavonoids are all indicated by the peak at 1366.08 cm⁻¹. The presence of C-CH₃ bending, nitro/sulfur molecule, gem dimethyl group, and myricetin is once again confirmed by the distinctive peak at 1456.99 cm⁻¹.

Table 3. *C. inerme* leaf extract from ethanol: FT-IR spectroscopy

Peak (cm ⁻¹)	Type of bonding	Functional groups	Flavonoids type
703.80	C-Hbending	Alkyne	Quercetin
895.15.86 sharp	C-H bending vibration	Aromatic substitution, gem distributed, olefinic group	Quercetin
1036.04	S=O stretching vibration	Sulfur compounds, sulfoxides, Thiocarbonyl group	Sodium Salts of Quercetin 5' Sulfonic Acid
1089.91	S=O stretching vibration	Sulfur compounds, Thio carbonyl group	Sodium Salts of Quercetin 5' Sulfonic Acid
1254.23	C-N Stretching	Aliphatic amine	
1366.08	C-N Stretching	Aromatic, sulphonamide, gem dimethyl group and Nitro compounds.	Myricetin
Strong			
1456.99	C-H bending	Alkanes	
1733.90	-C=C- Stretching	Alkenes	
2853.78	C-H Stretching vibration	Aldehyde	
2925.24	C-H Stretching	Alkanes	
3400.37	N-H Stretching	1°, 2° amines, Amides	

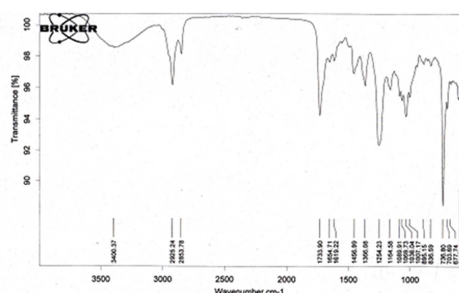


Fig. 3. *C. inerme* leaf extract from ethanol: FT-IR spectrum

Peaks at 1654.71 cm⁻¹ and 1733.90 cm⁻¹ in the FT-IR spectrum suggest the existence of the C-H bend, -C=C-Stretch, and the functional groups alkanes and alkenes. Peaks at 2853.78 cm⁻¹ and 2974.87 cm⁻¹ indicate the presence of aldehydes, alkanes, and C-H stretching vibrations. The comparable C-H stretch is at 2925.24 cm⁻¹. A distinct hump at 3400.37 cm⁻¹ corresponds to stretching vibrations of 1°, 2° amines, Amides, and N-H. Three different

types of flavonoids are found in the FT-IR spectra of an ethanolic extract of *C. inerme* (Bonjol) leaves: myricetin, quercetin, and Sodium Salts of Quercetin 5' Sulfonic Acid (Table 3, Fig. 3).

Conclusion

Preliminary data from the present study can be used to ascertain the chemical makeup of *Clerodendron inerme* leaves. The principal components that contribute to the therapeutic value of plants are chromophoric and functional groups, flavonoids, alkaloids, glycosides, fixed oil and lipids, phytosterols, terpenoids, phenolic compounds, and tannins. These bioactive chemicals' presence in plant extract attests to the plant's proper application in traditional medicine. This is also true when creating new medications by isolating particular compounds.

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