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Medicinal, Phytochemical Screening and Cytotoxicity of *Pergularia daemia* (Forssk.) Chiov.

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Abstract
Pergularia daemia (Forsk.) is a perennial twining herb belonging to Asclepiadaceae family grows widely along the roadsides. The whole plant possesses high medicinal value due to the presence of different bioactive compounds and traditionally used in treating various health problems. Keeping this in view, an attempt has been made to gather medicinal uses through literature survey, qualitative phytochemical screening of flowers and its toxicity to *Artemia salina*. Results revealed that it has diverse medicinal and pharmaceutical values. Flower extracts showed the presence of tannins, phenolic compounds, reducing sugars, carbohydrate, and amino acids. Aqueous extract of flowers showed cytotoxicity activity. The present study highlights the medicinal importance of flowers of *P. daemia*.

Key words: Perennial, traditional uses, pharmacological, phytochemistry, secondary metabolites

Introduction

The covet for the peculiar methods of treating various diseases has elevated globally majorly because of the cost-effect and the secondary complications raised due to the modern medications (Sridevi et al., 2015). Many peoples are switching towards the herbal medicine because of its safety, lesser cost, and the hope of complete cure of health problems. The medicinal plants are useful for healingas well as for curing health disorders because of the presence of the phytochemical constituents (Nostro et al., 2000). Research in medicinal plants and their applications has gained a renewed focus. The prime reason is that other system of medicine although effective and it has several side effectsthat often lead to serious complications (Pankaj et al., 2003). India is one of the world's leading producers of medicinal and aromatic plant products (Ananth et al. 2021). Plant based system of medicine being natural does not pose serious problems. Now-a-days there is widespread interest inevaluating drugs derived from plant sources (Doss and Anand, 2012). This interest primarily stems

from the belief that green medicine is safe and dependable, compare to costly synthetic drugs which are invariably associated with adverse effects (Nithyatharani and Kavitha, 2018). One such emerging plant is *Pergularia daemia* (Packirisamy and Moorthy, 2014) belonging to Asclepiadaceae (Khare, 2007) family which is already in the use as folk medicine in many ailments (Kathiswaran and Mrinalini, 2010; Sridevi et al., 2015). It is known as Uttaravaruni in Sanskrit and is widely distributedin the tropical and subtropical regions of Asia and South Africa and have multiple applications in different folk medicines (Packirisamy and Moorthy, 2014). Traditionally, it is used as anthelmintic, laxative, antipyretic, expectorant and used to treat diarrhoea and malarial fevers. Latex of this plant is used for toothache (Hebbar et al., 2004). Stem barks are used for cold & fever. Aerial parts of this plant have been reported for the various pharmacological activities like hepatoprotectivity (Kumar and Mishra, 2006), antifertility (Sadik et al., 2001a), antidiabetic (Wahi et al., 2002) analgesic, antipyretic and anti-inflammatory. Due to having multiple valuable properties; *P. daemia* is considered as an excellent phytomedicine (Bhaskar and Balakrishnan, 2009). It was noticed that flowers of *P. daemia* has less or no works in cytotoxicity using *Artemia salina*. Therefore, an attempt has been made to do qualitative analysis of phytochemical screening of flowers and gather the available pharmacological information on the plant.

Materials and methods

Collection of plant parts: The flowers of *Pergularia daemia* (Figure 1) were collected in August-September 2023 from nearby surroundings of Mahanadi River area in Cuttack district of Odisha, India. Plant was identified by the Authors.

Processing of collected flowers: The flowers of *P. daemia* are properly washed in tap water and then rinsed in distilled water. The rinsed flowers are shed dried and powdered using electrical blender. The powder was stored in air tight glass container, protected from sunlight until required for analysis.

Plant extraction: Aqueous extract of flowers is carried out using Soxhlet method (Marndi et al., 2024).

Phytochemical screening: Qualitative phytochemicals analysis was carried out using standard methods (Devi et al., 2023).

Tannins:1ml of extract was added to 3-5 drops of 0.1 % lead acetate, and the yellowish precipitate indicated the presence of tannins.

Saponins:1ml of extract was mixed with 2 ml of distilled water and then agitated in a graduated cylinder for 15 minutes. Formation of foam indicates the presence of saponins.

Figure 1: Flowers of *Pergularia daemia*

Flavonoids:1ml extract was added with 2 ml of 10 % NaOH solution and 2-3 drops of conc. H_2SO_4. A change to yellow colour which on addition of acid changed to colourless solution depicted the presence of flavonoids.

Terpenoids:1ml of extract was added with 1ml of chloroform and then 3-4 drops of conc. H_2SO_4 was added to it. Then a reddish-brown colour ring formation of interface indicates the presence of terpenoids.

Phenolic compounds:1 ml of filtrate was added with 3-5 drops of 1% ferric chloride solution. The formation bluish black colour indicates the presence of phenolic compounds.

Reducing sugar:1ml filtrate was added with 2 drops of Fehling's solution A and B respectively and kept in water bath for 5minutes. Formation of orange red precipitate indicates the presence of reducing sugar.

Alkaloids:1ml extract was added with 3 drops of Dragendroff's reagent and kept in water bath for some time. Formation of orange red precipitate indicates the presence of alkaloids.

Carbonyl compounds:1ml filtrate was added with 3-4 drops of 2,4-dinitrophenyl hydrazine reagent. Then the formation of yellow crystal indicates the presence of carbonyl compounds.

Carbohydrates: 1ml filtrate was added with Fehling's solution A and B, and kept for 5 minutes in water bath. If the red precipitation is formed, then it denotes the carbohydrate presence.
Lipid:1ml extract was added with 2-5 drops of Sudan-3. The appearance of pink droplet indicates the presence of lipid.

Protein:1ml extract was mixed with 2-3 drops of Millon's reagent and water bath for 3 minutes, the pink colour formation indicates the presence of protein.

Amino acid:1ml filtrate was added with 5 drops of Ninhydrin solution. Water bath till purple colour comes may be after 30 minutes, indicates the presence of amino acid.

Cytotoxicity analysis: For cytotoxicity test, hatching of Brine shrimp cysts was the initial process. Standardization of the optimum salinity was done at 2.0, 3.5 and 5.0 % saline concentration to check the optimum salinity for proper hatching of Brine shrimp cysts. The brine shrimp cysts are then incubated in 3.5 % saline water with proper aeration, room temperature (28-35 °C) or between and light for 72 hours. Different concentration (32 mg/ml, 63 mg/ml, 94 mg/ml and 125 mg/ml) of the aqueous extract of flowers were taken and 3.5 % of saline water was used to make the volume 2 ml in each test tube. Using standard methods the data was analysed (Devi et al., 2024).

Results and discussion

Literature survey revealed that plant parts of *P. daemia* have several pharmacological properties which are presented below.

Pharmacological activity

As a phytomedicine: The plant *Pergularia daemia* has been traditionally used as anthelmintic, laxative, antipyretic, expectorant and also used to treat infantile diarrhoea and malarial intermittent fevers (Chandak and Dighe, 2019). *P. daemia* is widely used by various tribal and rural communities in various regions of India, for the treatment of different health related problems, while predominantly the roots of the plant have been used to treat liver disease, and jaundice. This study aims towards medicinal properties, chemical constituents and other various important aspects of *P. daemia* (Bhaskar and Balakrishnan, 2009).

Antifertility and antioxidant activity: The ethanol extract of *Pergularia daemia* and its steroidal fraction are reported to have antifertility activity (Sadik et al., 2001b). In the preliminary phytochemical analysis, both aqueous and ethanolic extract indicated the presence of alkaloid, glucoside, steroid, flavonoid, saponin, terpenoid, tannin, and phenolic compound. The result obtained from the study revealed that *P. daemia* exhibited antioxidant activity which may be attributed to the presence of polyphenolic and other phytochemical constituents. This may be used in preventing oxidant stress related degenerated diseases (Shridevi, 2018).

Amelioratory activity: The whole plant *Pergularia daemia* extract was investigated for its Antiurolithiatic and diuretic activity (Chandak and Dighe, 2019), and the alcoholic extract of *P. daemia* exhibited significant diuretic activity as evidenced by increased total urine volume and the urine concentration. These findings were affirmed assertions made regarding the effectiveness of the extract of this plant species against urinary pathologies in the Indian folk medicine (Anagapann 2016).

Antibacterial activity: The ethanol extract *P. daemia* exhibited antibacterial activity. Some study report showed that the antibacterial activity of *Pergularia daemia* leaf extract was tested by using various solvents such as hexane, chloroform and ethyl acetate against B. subtilis, S. aureus, E. coli, and *P. vulgaris*. The promising antibacterial activity was observed in ethyl acetate and ethanol extracts of *Pergularia daemia* which showed significant antibacterial activity against S. aureus, P. aeruginosa, *A. hydrophila, E. coli*, and *S. typhi* (Senthilkumar et al., 2017; Ignacimuthu et al., 2009).

Anticancer activity: Anticancer activity of *Pergularia daemia* was screened and resulted that α-amyrin exhibited anticancer activity in low potency. Triterpenoids play a vital role as anticancer agents and structural modification of this class of compounds can result in the establishment of an innovative drug for the treatment of cancer (Devala, 2016).

Antidiabetic activity: Ethanol and aqueous extract of *Pergularia daemia* plant was investigated against alloxan induced hyperglycaemia. It significantly reduced blood glucose levels to normal which proved hypoglycaemic activity. This activity of *P. daemia* extract is possibly be due to the presence of β-sitosterol and quercetin. *P. daemia* on blood glucose level status in streptozotocin induced diabetic rats. The results suggested that oral administration of *P. daemia* possesses significant antidiabetic potential (Wahi et al., 2002).

Antiurolithiatic activity: The whole plant, *Pergularia daemia*, extract was investigated for its Antiurolithiatic and diuretic activity. Ethylene glycol feeding resulted in hyperoxaluria as well as increased renal excretion of calcium and phosphate. Alcoholic extract of *P. daemia* was given orally in curative and preventive regimens. Supplementation with extract significantly lowered the urinary excretion and kidney retention levels of oxalate, calcium, and phosphate. Furthermore, high serum levels of urea nitrogen, creatinine, and uric acid were significantly reduced by the extract (Vyas et al. 2013).

Anti-inflammatory, analgesic and antipyretic activity: Ethanol extract and its butanol fraction of *P. daemia* exhibited significant anti-inflammatory activity. This activity of *P. daemia* extract could be could be attributed due to the presence of steroids. The aqueous and ethanol extract of *P. daemia* showed the analgesic activity (Sutar and Pal, 2014) and the antipyretic activity was also reported from the aerial parts of *Pergularia daemia* extract (Usman, 2012).

Central nervous system depressant activity: The roots of *P. daemia* were evaluated for central nervous system depressant activity. Some experimental results concluded that both alcohol and aqueous extract showed central nervous system depressant activity and this activity mainly due to the presence of glycosides present in *P. daemia* roots (Chandak and Dighe, 2019).

Hepatoprotective activity: *Pergularia daemia* is traditionally used as a folk medicine for treating jaundice. A preliminary investigation on the aerial parts of *P. daemia* showed significant hepatoprotective activity (Vyas et al. 2013).

Phytochemical screening

The preliminary phytochemical analysis of aqueous extract of *P. daemia* flowers revealed the presence of carbohydrates, tannins, flavonoids, amino acid, reducing sugar, and phenolic compounds (Table 1). Due to the presence of multiple bioactive phytochemical compounds in *P. daemia*, resulted as an excellent phytomedicine possesses various therapeutic properties against several health problems. The cytotoxicity analysis revealed that highest death rate percent was observed at 125 mg/ml concentration (Table 2; Figure 2). It indicates that flower extracts of *P. daemia* can be used for further advance experimental works in cancer research.

Table 1: Phytochemical analysis for primary and secondary metabolites of aqueous extract of flowers of *P. daemia*

Metabolites	Test	Results
Tannins	Lead test	Present
Saponins	Foam test	Absent
Flavonoids	Ferric chloride	Absent
Phenolic compounds	Ferric chloride	Present
Reducing sugar	Fehling's test	Present
Alkaloids	Dragendroff's reagent test	Absent
Carbonyl compounds	2,4-DNP test	Absent
Carbohydrate	Fehling's test	Present
Lipid	Sudan-III	Absent
Protein	Millon's test	Absent
Sucrose	Iodine test	Absent
Amino acid	Ninhydrin test	Present

Table 2: Cytotoxicity analysis of aqueous extract of flowers of *P. daemia*

Extract	Concentration (mg/ml)	Initial number of Nauplii	Number of deaths of Nauplii (After in hours)				Death rates (%)
			1	2	3	4	
Aqueous	32	10	0	0	1	1	10
	63	10	0	0	1	2	20
	94	10	0	0	1	3	30
	125	10	0	1	3	4	40
	3.5 % saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100

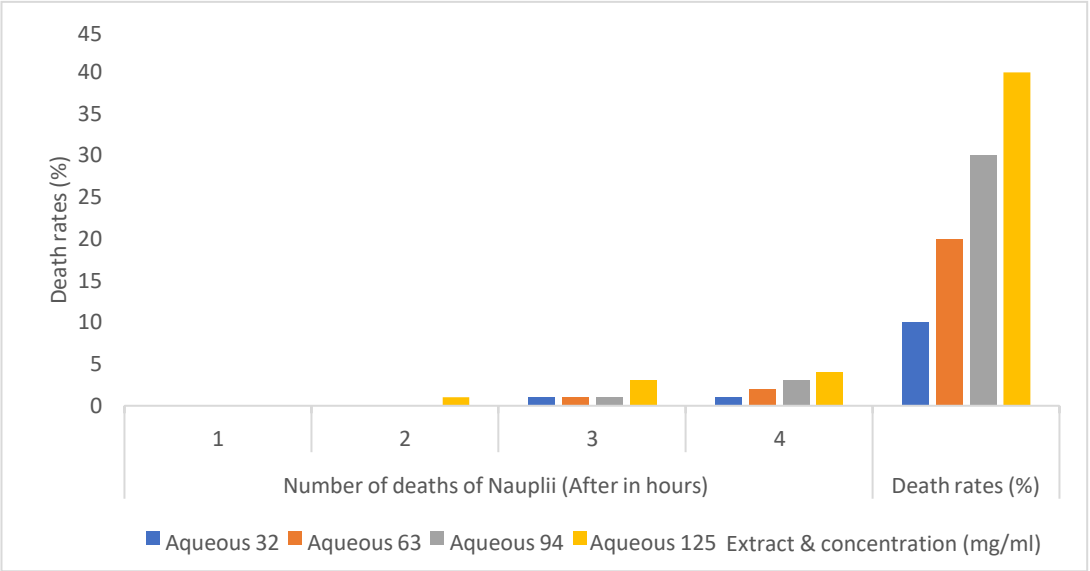

Figure 2: Death rate (%) of aqueous extract of flowers against *Artemia salina*

Conclusion

The present study concludes that *Pergularia daemia* is used to cure several health problems and reported diverse pharmacological values. Flowers has less reports and the present study highlights its phytochemicals and cytotoxicity potentials.

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