VOLUME 10 ISSUE 2 2024

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 10, No. 2, 844-851 (2024)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Hot-Water Extract of *Mussaenda luteola* Flowers – A Source of Antioxidant, Anti-Inflammatory and Antimicrobial Agents

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Received: 12th September, 2024; **Accepted:** 21st September, 2024; **Published online:** 23rd September, 2024

https://doi.org/10.33745/ijzi.2024.v10i02.082

Abstract: In the present study, antioxidant, anti-inflammatory and antimicrobial potentials of hot-water extract of *Mussaenda luteola* (family Rubiaceae) flowers were investigated. It is an ornamental plant grown in tropical countries, commonly known as Vellaiilai in Tamil and used in the treatment of jaundice, asthma, ulcers, leprosy, diuretic, hypolipidemic effect, hepato-protective, and many others. Phytochemical analysis of hot-water extract of *M. luteola* flower showed the presence of tannin, flavonoids, alkaloids, saponins, steroids and triterpenoids. The extract displayed an excellent total phenolic and total flavonoid contents. Results of antioxidant studies revealed that the hot-water extract of *M. luteola* flower possessed an efficient 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging potential, and it also exhibited anti-inflammatory potential and dose dependently (50, 100 and 150 µg/ml) inhibited the heat induced lysis of the human red cell membrane. Furthermore, the *M. luteola* flower extract also demonstrated antimicrobial activities against Gram (+) and Gram (−) bacteria and significantly inhibited the bacterial strains *Escherichia coli* (ATCC 25923) and *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 25619) in well diffusion method. Therefore, the results indicate that the flower of *M. luteola* can serve as a potential antioxidant, anti-inflammatory and antimicrobial agentin food and pharmaceutical industries.

Keywords: *Mussaenda luteola*, Hot-water extract, Antioxidant, Anti-inflammatory, Antimicrobial, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*

Citation: Madhiyazhagan Pavithra, Perumal Sarath, Kiran Sharma B. and Kandasamy Sasikumar: Hot-water extract of *Mussaenda luteola* flowers – A source of antioxidant, anti-inflammatory and antimicrobial agents. Intern. J. Zool. Invest. 10(2): 844-851, 2024.

https://doi.org/10.33745/ijzi.2024.v10i02.082

Introduction

Plants represent one of the most important resources of nature, providing humankind with the primary source for various phytochemicals used in human and animal nutrition. Indeed,

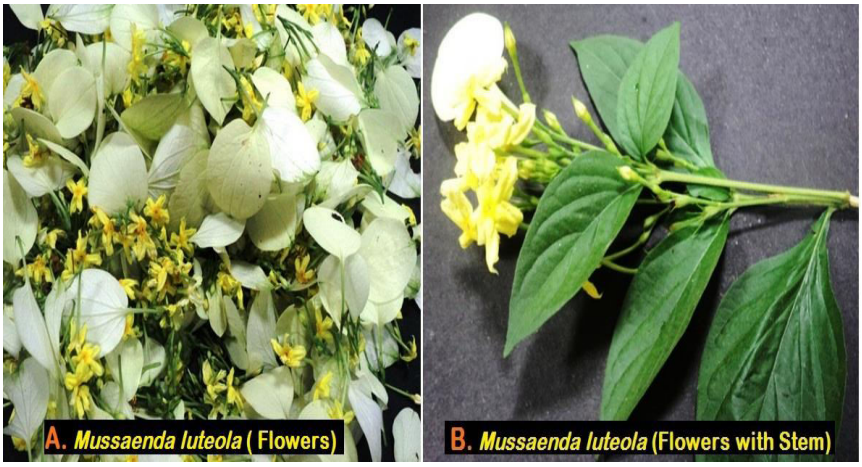

Fig. 1: *Mussaenda luteola* bright yellow flowers with creamy colour corolla

plants have the extraordinary capacity to synthesize a very great number of low molecular weight organic substances called secondary metabolites, usually with a specific and complex structure. Since 1990s the phytochemistry of *Mussaenda* species were studied (Huxley *et al*., 1997). This plant is increasingly popular for their showy colour all throughout the year in South Florida landscapes. They are members of the Rubiaceae (coffee family) and are native from West Africa through the Indian sub-continent, South East Asia and Southern China. There are more than 200 known species, out of which ten are found in cultivation and three being widely used for landscaping. *Mussaenda luteola* has flat-topped flower clusters (corymbs), with bright yellow corollas and a single enlarged calyx lobe that is yellow to cream (Fig. 1). Some species have historically been used in Chinese and Fijian traditional medicine as a diuretic, antipyretic, expectorant, jaundice, asthma and antimicrobial (Vidyalakshmi *et al*., 2009). The phytochemicals present in the plant are polyphenols, triterpenoids, flavonoids, tannins, catechins, vitamins C and E, β-carotene and several others which are responsible for health protective benefits. The mixture of these compounds may provide better protection than single phytochemical due to their synergistic effects (Alissa *et al*., 2017).

ROS play important role in the host defense mechanisms and excessively produced ROS from phagocytic cells including polymorphonuclear leukocytes and mononuclear cells creates oxidative stress in the cells and deregulate the cellular functions causing cellular and tissue damage, which cause inflammatory diseases, cancer and infectious condition (Schafer *et al*., 2000). As antioxidants terminate ROS radical-mediated oxidative reactions directly, they might be used to prevent cancer, neurodegenerative and inflammatory diseases (Wang *et al*., 2024). The antioxidant potential of compounds is due mainly to their redox properties, which allow them to act as reducing agents or hydrogen atom donors. Thus, natural antioxidants function as free-radical scavengers and chain breakers, complexes of prooxidant metal ions and quenchers of singlet-oxygen formation (Zar Kalai *et al*., 2024).

Antibiotic resistance is a problem that continues to challenge the healthcare sector in a large part of the world in both developing and developed countries. It raises the demand for finding new alternative potent antimicrobial agents. Nowadays the therapeutic properties of plant polyphenols have also demonstrated antimicrobial effects by causing structural or functional damage to the bacterial cell membrane. Several studies have also shown that extract rich in polyphenols exert both antioxidant and antimicrobial effects (Garcia-Manriquez *et al*., 2024). Five new triterpenoid saponins (Du *et al*.,

2024) derivatives of heinsiagenin A, A new cycloartane-type saponins (Mai *et al*., 2024), two new monoterpenoidgluco-indole alkaloids, 7α-morroniside, 7-epiloganin, (7β)-7-O-methyl-morroniside, 5(S)-5-carboxystrictisidine and apigenin-7-O neohesperidoside were isolated from the aerial parts of *M. luteola*. However, the flower of *M. luteola* has not been exposed to phytochemical and pharmacological investigations. Therefore, the present study aimed to perform phytochemical screening and also to evaluate the *in vitro* antioxidant, anti-inflammatory and antimicrobial potentials of the hot-water extract of the *M. luteola* flowers.

Materials and Methods

Preparation of extracts:

M. luteola flowers were collected from horticulture garden, Vellore, Tamil Nadu, India. The flower was pale yellow in colour and it was shade-dried, pulverized to powder in a mechanical grinder. 10 g powder was successively extracted with water and extracts were concentrated under reduced pressure in a rotary evaporator and then dried. The extracts thus obtained were used directly to assess the antioxidant, anti-inflammatory and antimicrobial potentials through various *in vitro* assays.

Phytochemical studies:

Preliminary qualitative phytochemical analysis was carried out to identify the secondary metabolites present in the hot-water extracts of *M. luteola* flower (Harborne *et al*., 1984; Trease *et al*., 2002).

Quantification of total phenolics and flavonoids contents:

The total phenol content of *M. luteola* flower extracts were determined using Folin -Ciocalteu reagent by the method of Siddhuraju and Manian (2007). Extract at concentrations of 50 µg/ml and 100 µg/ml was taken into test tubes separately. 1 ml of 1 N Folin-Ciocalteu phenol reagent and 2.5 ml sodium carbonate solution (20%) were added sequentially in each tube. After that the test tubes were placed in dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. Gallic acid was used as the standard and the results are expressed as gallic acid equivalents (GAE).

The flavonoid content was estimated using aluminium chloride method described by Zhishen (1999). 0.5 ml of flower extract aliquot at concentrations of 50 µg/ml and 100 µg/ml was mixed with 2 ml distilled water separately and subsequently with 0.15 ml of 5% $NaNO_2$ solution. After 6 min, 0.15 ml of 10% $AlCl_3$was added and the mixture was allowed to stand for 6 min, and then 2 ml of 4% NaOH solution was added to the mixture. Distilled water was immediately added to bring the final volume to 5 ml, and then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm against the water blank. Quercetin was used as the standard and the results are expressed as quercetin equivalents (QE).

Anti -Oxidant (*DPPH radical scavenging*) *Assay*:

The antioxidant activity of *M. luteola* flower extract and standard (Ascorbic acid) was measured in terms of hydrogen donating ability using a stable, commercially available organic and nitrogen centered DPPH radical (Brand-Williams *et al.*, 1995). 1 ml of extract was mixed with 3 ml methanol containing DPPH (0.025 g/l) and incubated in the dark for 30 min. The absorbance was measured at 515 nm with UV–Visible spectrophotometer. The standard was prepared in the range of 0–2.5 mmol/l. The inhibition rate of DPPH radical scavenging activity was calculated as per below given formula.

$$\% \text{ of Inhibition} = \frac{\text{OD of Control} - \text{OD of Sample}}{\text{OD of Control}} \text{ X } 100$$

Anti- Inflammatory (*HRBC -Membrane Stability*) *Assay*:

Releases of extracellular enzymes like lysosomal enzymes are responsible for acute or chronic inflammation. The non-steroidal drugs act

either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane (Chowdhury *et al*., 2014). The various concentrations range from 50 – 150 µg/ml of flower extracts to predict the *in vitro* anti-inflammatory potential. Blood was collected from healthy volunteers and mixed the blood with equal volume of Alsever solution and centrifuged with isosaline. To 1 ml of HRBC suspension equal volume of test drug was added. All the assay mixtures were incubated at 37°C for 30 min and centrifuged. The haemoglobin content in the supernatant solution was estimated by using spectrophotometer at 560 nm (Saleem *et al*., 2011). The percentage of protection was calculated by given formula below:

$$\% \text{ of Protection} = \frac{\text{OD of Control} - \text{OD of Sample}}{\text{OD of Control}} \text{ X } 100$$

Antimicrobial (*Well diffusion*) *method*:

McFarland standard solution was used to standardise the density of bacterial suspensions used in this study.The hot-water extracts of *M. luteola* flower were tested against the pathogenic micro-organisms including *Staphylococcus aureus* (G+) (ATCC 25923), *Pseudomonas aeruginosa* (G+) (ATCC 25619) and *Escherichia coli* (G-) (ATCC 25923). Bacterial strains were cultured for 24 h at 37°C on nutrient agar (NA, HiMedia, India),

Briefly, 50 µl of suspension containing approximately 10^{-8} colony forming units/ml of dilution of bacterial cells were swabbed on to MHA plates. Wells (6 mm diameter) were made in each plate with sterile cork-borer and 100 µl of various concentrations (50, 100 and 200 mg/ml) of flower extracts were added into the wells and allowed to diffuse at RT for 2 h. For the positive control, amikacin (30 µg/disc) for Gram negative bacteria and vancomycin (30 µg/disc) for Gram positive bacteria was used. Negative controls were prepared using the same solvents employed to dissolve the flower extracts. Plates were incubated at 37°C for 18–24 h for bacterial strains. Antimicrobial activity was assessed by measuring the diameter of the growth inhibition zone (IZ) in millimetres (including the well diameter of 6 mm) for the test organisms compared to controls (Perez *et al*., 1990).

Statistical analysis:

Statistical analysis was carried out with Graph Pad Prism 6 software (Graph Pad Software, Inc., USA), and results are expressed as mean ± standard deviation.

Results

Phytochemical analysis of hot-water extract from flowers of *M. luteola* was carried out, and it presented a green flag to several secondary metabolites, such as alkaloids, saponins, flavonoids, terpenoids, sterols, and tannins. The results are given in more detail in Table 1.

From Table 2, it can be seen that the calibration curve gave the total phenolic content of the hot-water flower extract as 245.96 ± 5.50 mg gallic acid equivalents/g and that of flavonoids as 223.95 ± 4.277 mg rutin equivalents/g.

The changes over time in the free radical reduction capacity of microemulsion systems are indicated by a reduction in the absorbance of the DPPH sample. This formula is utilized to determine the percentage of radical scavenging. According to the DPPH assay, a widely used method for the determination of free radical scavenging because of its simplicity, the extract exhibited high antioxidant activity. The flower extract demonstrated DPPH radical scavenging activity of 78.69 ± 0.52% at a concentration of 500 µg/ml, while the control-as ascorbic acid-was 89.06 ± 0.43% (Table 3).

The HRBC membrane stabilization has been used as a method for preliminary screening of *in vitro* anti-inflammatory activity. The hot-water extract of *M. luteola* flower showed significant % of protection was 79.99±1.315 and the standard diclofenac sodium was 89.16±1.301 at the concentration of 150 µg/ml. The anti-inflammatory activity increased as the extract concentration was increased (Table 4).

The resulting antimicrobial activity is presented in Table 5. The extracts demonstrated

Table 1: Phytochemical Analysis of hot-water extract of *Mussaenda luteola* flower

S. No.	Phytochemical Test	Inferences
1	Aleurone Grains	+
2	Alkaloids	+
3	Carbohydrates	-
4	Fat and Fixed Oils	+
5	Flavonoids	+
6	Tannin	+
7	Proteins	-
8	Saponin Glycosides	+
9	Steroids	+
10	Triterpenoids	+
11	Napthoquinones	+

= Present; - Absent

Table 2: Total phenolics and flavonoid contents of hot-water extract of *M. luteola* flower

Solvent Extract	Total Phenolics (mg GAE/g extract)	Flavonoids (mg QE/g extract)
Hot-Water	245.96±5.501	223.95±4.277

Table 3: Antioxidant potentials of hot-water extract of *M. luteola* flower

Concentration (µg/ml)	DPPH	
	Ascorbic Acid	Hot-Water Extract
50	77.26±1.053	67.09±2.588
100	83.34±1.315	74.92±2.694
150	89.06±0.431	78.69±0.523

Table 4: Anti-inflammatory potentials of hot-water extract of *M. luteola* flower

Concentration (µg/ml)	% Stabilisation of HRBC	
	Diclofenac	Hot-Water Extract
50	72.59±1.880	67.61±0.919
100	82.71±0.997	74.62±1.060
150	89.16±1.301	79.99±1.315

Table 5: Antimicrobial activity of hot-water extract of *Mussaenda luteola* flower

Samples	Concentration (µg/ml)	Zone of Inhibition in diameter (mm)		
		Escherichia coli (ATCC 25922)	*Staphylococcus aureus* (ATCC 25923)	*Pseudomonas aeruginosa* (ATCC 25619)
Amikacin	30µg per disc	24	21	23
Hot-Water Extract	5	14	15	13
	10	17	16	15
	20	21	19	19

considerable inhibitive action against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, showing a meaningful comparative effectiveness when tested against the standard antibiotic, amikacin.

Discussion

The plant *Mussaenda luteola* that is taken under investigation showed the presence of alkaloids, quinone, terpenoids, oils and fats. Steroids, flavonoids and phenols are found abundantly in the current analysis and the total content of those were estimated in the flowers of *Mussaenda luteola*. The phenol content of a plant depends on a number of intrinsic (genetic, extracting solvent) and extrinsic (environmental, handling and development stage) factors (Olas *et al.*, 2024). Phenolic compounds from plants constitute one of the major secondary metabolites having redox properties, which allow them to act as antioxidants (Bonta *et al.,* 2020). Flavonoids are one of the major phenolic compounds present in the extract and their antioxidant potentials depends on the presence of free OH groups, especially 3-OH (Tawfik *et al.,* 2024). Super-oxide is a reactive oxygen species that can damage cells and DNA, leading to various diseases. The determination of total phenolic concentration could be used as a basis for rapid screening of antioxidant potentials facilitated by their hydroxyl groups (Azmoonfar *et al*., 2024). The high phenolic and flavonoid content is responsible for the bioactivity of these crude extract (El-Saadony *et al.*, 2024). These results show that the potential scavenging abilities of phenolic substances might be due to the active hydrogen donor ability of hydroxyl substitution. High molecular weight and the proximity of many aromatic rings and hydroxyl groups are more important for the free radical-scavenging activity by tannins than their specific functional groups (Ben *et al.,* 2024).

The HRBC membrane stabilization implies that the extract may well stabilize the lysosomal membranes because the erythrocyte membrane is analogous to the lysosomal membrane (Pandey *et al.,* 2024). Stabilization of lysosomal is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bacterial enzymes and proteases, which causes further tissue inflammation and damage upon extra-cellular release. The extra-cellular activity of these enzymes is said to be related to acute or chronic inflammation. The non-steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane (Kavitha *et al*, 2024).

The antimicrobial activity of the extract may be attributed to the high content of flavonoids, which have been reported to be involved in inhibition of nucleic acid biosynthesis and other metabolic processes (Biharee *et al*., 2020). Moreover, flavonoids are synthesized by plants in response to microbial infection. Phenolic compounds with a C-3 side chain at a lower level of oxidation and containing no oxygen have often been reported to be antimicrobials (Berkada *et al*., 1978). The mechanism of the toxicity of polyphenols against microbes may be related to inhibition of hydrolytic enzymes (proteases) or other interactions that inactivate microbial adhesions, cell envelope transport proteins and non-specific inter-actions with carbohydrates (Maghsoudi *et al*., 2024). Isolation of the responsible bioactive compound is necessary for fully elucidating the antimicrobial activity of these crude extracts. This might also provide insight about their possible used in food and non-food systems. The results of this study support the use of these plants for human and animal disease therapy and reinforce the importance of the ethnobotanical approach as a potential source of bioactive substances. The higher plants exhibits antimicrobial potentials due to the presence of naturally occurring antimicrobial compounds (Cowan *et al*., 1999) like flavonoids, phenolics, catechols, marmalosin, furocoumarin, alkaloids, tannins and other secondary metabolites.

Conclusion

The plant –based antimicrobials, anti-inflammatory agents represent a vast untapped

source of medicines and which poses little or no side effects. Our results suggest that *M. luteola* is a potential source of antioxidant, anti-inflammatory and antimicrobial agents and could be used as a natural antioxidant and preservative in food and non-food systems. In developing, countries where medicines are quite expensive, investigation on antimicrobial activities from ethnomedicinal plants may still be needed. Further phytochemical analysis is required to isolate the bioactive compounds from the flower extract that show a broad spectrum of pharmacological activity. It is obvious that these phytochemicals will find their way in the arsenal of antimicrobial, anti-inflammatory drugs prescribed by physicians**.**

Acknowledgements

The authors are grateful to the management of SS Herbals, Dharmapuri, VIT University, Vellore and DDGD Vaishnav College, Chennai for the support in pursuing research.

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