Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol.14 , Issue 33s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Systematic Extraction, Isolation, and Phytochemical Investigation of Various Parts of Murraya paniculata (L.) Jack

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Cite this paper as: Abhishek Sharma, Omprakash Goshain (2025) Systematic Extraction, Isolation, and Phytochemical Investigation of Various Parts of Murraya paniculata (L.) Jack. Journal of Neonatal Surgery, 14, (33s) 961-966

ABSTRACT

Murraya paniculata (L.) Jack is a member of the Rutaceae family, an aromatic evergreen medicinal shrub generally distributed throughout tropical and subtropical Asia, India, China, Thailand Malaysia and Indonesia. Various plant parts have been utilized in the management of inflammation, diabetes mellitus, microbial infections, gastrointestinal disorders, neurological ailments and pain syndromes used for centuries in diverse ethnomedicinal traditions including but limited to ayurveda. siddha and traditional Chinese medicine. This paper reports a systematic and comprehensive exploration of bioactive phytoconstituents in five different plant parts of M. paniculata via extraction, isolation, chromatographic separation and structural elucidation. Conventional maceration, Soxhlet extraction, ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE) were used for multiple extract techniques with solvents of varying polarity (petroleum ether, chloroform, ethyl acetate, methanol and water). (TLC), and reverse-phase high-performance liquid chromatography (RP-HPLC) resulted in compounds such as carbazole alkaloids, coumarins, flavonoids, and a triterpenoid. Ultraviolet-visible (UV-Vis) spectroscopy, Fourier transform infrared (FTIR) spectroscopy, proton and carbon nuclear magnetic resonance (1H/13C NMR) spectroscopies, and high-resolution mass spectrometry techniques were used to confirm the structural characterization. Integrated phytochemical-pharmacological profile disclosed on these findings prunes M. paniculata as an encouraged candidate for pharmaceutical drug discovery and nutraceutical development in the genesis of alternatives that validate its traditional uses.

KEYWORDS: Murraya paniculata; carbazole alkaloids; coumarins; flavonoids; essential oils; phytochemical investigation; extraction techniques

INTRODUCTION

Medicinal plants have been the basis of traditional and modern pharmaceutical research, and about 50% of all all clinically approved drugs were derived from a medicinal plant extrinsically or as structural scaffolds [1]. Among this vast pharmacopoeia, the family Rutaceae is well known for its varieties of phytochemistry, especially alkaloids, terpenoids, flavonoids, and aromatic coumarins [2]. Although nine to twelve true species are accepted in the genus Murraya, which was originally formally described by Linnaeus and named in honour of Swedish botanist Johan Andreas Murray [3], the most phytochemically investigated and ethnobotanically important among them is Murraya paniculata (L.) Jack (synonym M. exotica L.) [3].

Murraya paniculata, often referred to as orange jasmine, Chinese box, or Kamini in Ayurvedic literature is a small-to-

moderate sized evergreen shrub or tree growing to 2–4 m tall with glossy pinnate leaves and fragrant white paniculate flowers followed by very conspicuous orange-red ovoid berries [4]. The species is indigenous to South and Southeast Asia, with established populations in India (Himalayan foothills through Kerala), Sri Lanka, southern China, Thailand, Malaysia, the Philippines, and Indonesia; it has been introduced in Australia, parts of Africa and the Caribbean [5]. That broad ecological plasticity allows the plant to grow under a wide range of environmental conditions, as it thrives in tropical and subtropical environments (mean annual temperature 25–35°C) with soils with pH values between 5.5 and 7.5 [6]..

In many traditional medicine systems, different M. paniculata organs perform different therapeutic functions. The leaf decoction is a medicinal remedy in Ayurveda for rheumatism, diabetes mellitus and dysmenorrhoea; the root bark is employed against dysentery, malaria and fever; flowers are used as anxiolytics and cephalic agents; fruits are used in digestive preparations [7]. Plant is used for skin diseases in Siddha system and its root is used in Traditional Chinese Medicine (TCM) to detoxification and anti-inflammatory management [8]. In Thailand, a quantitative ethnobotanical survey list M. paniculata preparations as part of 65% of therapeutic repertoire use by traditional healers for skin infection and inflammatory conditions [9].

The pharmacological properties of M. paniculata are attributed to a complex and structurally varied phytochemical matrix. More than 200 secondary metabolites from different plant parts have been recorded [10]. [11]. Flavonoids, and triterpenoids [12, 13]

Although a large body of ethnopharmacological and phytochemical research exists regarding M. paniculata, the coverage is quite scattered due to the individual study of single plant parts or chemical classes. There is a blatant lack of a holistic, multi-organ phytochemical characterisation that embraces contemporary extraction optimisations, stringent chromatographic isolation, expansive spectroscopic elucidation and correlative pharmacological evaluation. Moreover, comparative quantitative information on natural product diversity among plant parts—imperative for guiding organ-specific drug discovery—is sparse and methodologically inconsistent across studies [14].

Thus, the current study was conducted to: (i) provide a systematic comparison of conventional versus advanced extraction methods on all five plant organ types (leaves, bark, roots, flowers and fruits); (ii) isolate and structurally characterise main constituents responsible for bioactivity using multi-spectroscopic tools; (iii) construct quantitative phytochemical profiles for each organ type. The dataset integrated hereby is expected to bolster the pharmaceutical valorisation of M. paniculata with a solid scientific platform and to inform future bioassay-guided fraction approach and clinical translation efforts [15]

MATERIALS AND METHODS

2.1 Collection, Authentication and Voucher Deposition of Plant Material

Mature leaves, stem bark, root bark, open flowers and ripe fruits of fresh plant material were obtained during pre-flower and flowering season (February to April) from authenticated in Uttar Pradesh, India. Only morphologically intact and disease-free specimens were chosen; contaminated or mechanically damaged material was discarded. All five plant parts were collected from the same individual plants separately to eliminate inter-plant variability.

2.2 Preparation of Plant Material

Part of each plant was cleaned in running tap water and then in distilled water to remove surface contaminants, and shade-dried at 28–32 °C for 14–21 days until constant weight. Root and bark samples were further scraped to remove surface cork. Subsequently, all materials were pre-dried in an oven at 45°C for 48 h until moisture contents had reached uniform values below 8% . The dried materials were ground to a consistent coarse powder (40-mesh sieve) with the help of a Wiley mill (Remi Equipment, India). Powdered samples were stored at 4 °C in the dark, under vacuum desiccation of silica gel, to minimise oxidative degradation before use.[16][17]

2.3 Extraction Procedures

2.3.1 Sequential Soxhlet Extraction

For each part 50 g powdered plant material was subjected to sequential Soxhlet extraction (Borosil, India) with solvents of increasing polarity: petroleum ether (60–80°C, 6 h), chloroform (61°C, 8 h), ethyl acetate (77°C, 8 h) and methanol (65°C, 10 h). Each solvent cycle was executed following volatile removal via air-drying. The extracts were concentrated under reduced pressure at temperatures of below 50°C using rotary evaporator (Buchi R-300, Switzerland) and weighed

gravimetrically to determine percentage yield [18].

2.3.2 Maceration

A duplicate aliquot of each powdered plant fraction (50 g) was extracted with 80% methanol (500 mL, 1:10 w/v) in amber stoppered conical flasks at room temperature (25 ± 2°C) for 72 hours while being stirred every 12 hours. Whatman No. 1 filter paper was used for filtration and marc was re-macerated twice for 48 h each time. Recombined filtrates were pooled and concentrated under vacuum at 45 °C. Aqueous decoctions were also prepared by boiling the plant material (1:10 w/v) and filtering after cooling for 30 minutes [19].

2.3.3 Ultrasound-Assisted Extraction (UAE)

UAE was done using an ultrasonic bath (Elmasonic S 60, Germany) with frequency at 40 kHz. Powdered plant material (10 g) was added into 80% ethanol (100 mL) in a sealed 250 mL Erlenmeyer flask inside the ultrasonic bath at the temperature of 45°C for 45 min. Extraction vessel temperature was recorded with a thermocouple and held within ±2°C of set point. The suspension was immediately filtered and concentrated after the sonication. This was done three times per plant part and average yield values were calculated [20].

2.3.4 Microwave-Assisted Extraction (MAE)

MAE was performed with a focused microwave system (CEM Discover SP, USA) at 500 W in 80% methanol of a 50 mL extract per 5 g plant material. Temperature was increased to 80°C for 2 min and held for 10 min Before cooling passively to room temperature. The extract was filtered using a 0.45 µm nylon membrane filter and tilted down to a concentration of 10 times of the original solution things were provided. In preliminary tests, microwave power, solvent-to-material ratio, irradiation time and temperature of extraction were optimised using a single factor method [21].

2.4 Fractionation by Liquid-Liquid Partitioning

Ten gram of each crude methanolic extract derived from different plant parts was suspended in distilled water (100 mL) and successively fractionated with petroleum ether, chloroform and ethyl acetate (3 × 100 mL) using a separatory funnel. All the organic fractions were dried over anhydrous sodium sulphate, filtered and evaporated under reduced pressure to get respective petroleum ether (non-polar terpenes/fats), chloroform (alkaloids, aglycones), ethyl acetate (polyphenols, flavonoids) and residual aqueous (polar glycosides, saponins) fractions. Fractional yields were determined and each fraction was preliminary screened of its phytochemical profile by TLC.

2.5 Column Chromatography and Compound Isolation

Silica gel 60 (70–230 mesh, Merck) was used for a column chromatography with silica gel of 60 cm × 3.5 cm (inner diameter) size for each part of the plant in chloroform and ethyl acetate fractions. Following this, hexane:ethyl acetate (in stepwise gradient 100:0 → 0:100 v/v) and ethyl acetate:methanol gradients were used для градиентного элюирования. In 25 mL fractions were collected, and similar fractions pooled based on TLC monitoring (hexane:EtOAc, various ratios; detection by UV254/365 nm & Dragendorff/$KMnO_4$ sprays). Depending on intended use, pooled fractions were further purified by preparative TLC or Sephadex LH-20 gel filtration (methanol/chloroform, 1:1) [24]. Analytical RP-HPLC confirmed the final purity of isolated (≥98%) compounds.

2.6 Spectroscopic Characterisation

UV-Vis spectra (200–500 nm) were recorded in methanol using a Shimadzu UV-2600 spectrophotometer. FTIR spectra (KBr disc) were acquired on a Bruker ALPHA II spectrometer, operating within the range of 4000–400 cm^{-1}, with 32 scans at a resolution of 4 cm^{-1}. 1H NMR (400 MHz) and 13C NMR (100 MHz) spectra were obtained on a Bruker AVANCE III 400 spectrometer in $CDCl_3$ or DMSO-d_6; chemical shifts are reported relative to TMS (δ = 0 ppm). HR-ESI-MS was carried out on a Waters Xevo G2-XS QTof instrument in positive and negative ionisation modes respectively.

2.7 Qualitative Phytochemical Screening

Qualitative phytochemical screening used the well documented methods described by Harborne, using Dragendorff's reagent (alkaloids), Shinoda test (flavonoids), UV fluorescence (coumarins), foam test (saponins), ferric chloride test (tannins/phenolics) and Liebermann-Burchard reaction (sterols/triterpenoids).

2.8 Statistical Analysis

All experiments were performed in triplicates, unless otherwise stated. Data are presented as mean ± SD. Statistical analyses were executed using GraphPad Prism 9.0. One-way ANOVA with Tukey's post-hoc test was used to assess significant differences between groups; $p < 0.05$ was considered statistically significant. IC_{50} values were derived from nonlinear regression (sigmoidal dose-response model). Pearson correlation was employed to assess relationships between phytochemicals content and pharmacological activity.

RESULTS

3.1 Extraction yield by method and plant part

Extraction outcomes differed significantly with respect to each solvent/method used and plant part examined. All methods and plant part quantitative yield data are shown in Table 1. Assessed over five extraction methods, UAE with 80% ethanol provided the highest yield for all plant organs tested - leaf fraction (20.2 ± 1.6% w/w), roots (17.3 ± 1.5%), bark (14.4 ± 1.3%), flowers (12.8 ± 1.3%) and fruits (data not shown). Yield for conventional maceration with methanol was comparable but slightly lower (leaves: 15.6 ± 1.4%) and yields from Soxhlet extraction using ethanol were reported at 14.1 ± 1.2% (from leaves). Overall, leaves showed the highest total extractable matter of all non-volatile components tested; while flowers had the highest essential oil yield. One explanation for UAE's superior potential is related to the ability of ultrasound waves to induce cavitation effects that weaken cell walls as solvent can penetrate more easily, thus leading to greater release of intracellular secondary metabolites in a more effective manner compared to thermal or static methods.

Table 1. Comparative extraction yield (% w/w, mean ± SD, n = 3) of Murraya paniculata plant parts using different extraction methods

Extraction Method	Leaves (% w/w)	Bark (% w/w)	Roots (% w/w)	Flowers (% w/w)
Maceration/MeOH	15.6 ± 1.4	13.1 ± 0.8	13.7 ± 1.0	10.2 ± 0.7
Soxhlet/EtOH	14.1 ± 1.2	11.7 ± 0.6	12.1 ± 1.4	8.6 ± 0.8
UAE/EtOH (40 kHz)	21.2 ± 1.6	14.4 ± 1.3	17.3 ± 1.5	12.8 ± 1.3
MAE/MeOH (500 W)	17.9 ± 1.5	12.9 ± 1.2	15.8 ± 1.2	11.5 ± 0.8
Aqueous decoction	10.9 ± 0.8	7.5 ± 0.7	11.3 ± 0.6	9.7 ± 0.5

3.2 Qualitative and Quantitative Screening of Phytochemicals

Results of qualitative phytochemical screening from extracts of all five plant parts are shown in Table 2. Alkaloids were found across the organs with the most plentiful concentrations in leaves and roots, aligning with the above observation of carbazole alkaloid biosynthesis being biased towards these organs. Carbazole-exclusively recognition (Dragendorff-positive, yellow-orange sediment) was strongest in leaf and root reads, absent in blooms and fruits. Flowers and leaves had the maximal contents of flavonoids. Coumarins, identified through distinctive blue-violet UV fluorescence at 365 nm (before and after alkali treatment), were found to be most abundant in bark and roots—matching the previously known occurrence of scopoletin and umbelliferone within these tissues. These secondary metabolites presented organ specific abundance with high accumulation of tannins in bark and high saponin levels in roots.

Table 2. Qualitative phytochemical screening results for Murraya paniculata extracts by plant part. +++: abundant; ++: moderate; +: trace; −: absent

Phytochemical Class	Leaves	Bark	Roots	Flowers	Fruits
Alkaloids	+++	+++	+++	+	+
Carbazole Alkaloids	+++	++	+++	-	-
Flavonoids	+++	++	++	+++	++
Coumarins	++	+++	++	+	+
Terpenoids	++	+++	+++	++	++
Tannins	++	+++	+	+	+
Saponins	+	++	+++	+	+
Essential Oils	+++	++	++	+++	++
Steroids	+	++	++	-	+
Glycosides	++	+	+	++	+

DISCUSSION

The current study provides a detailed and systematic characterisation of the phytochemical profile of five organelle types, anatomically and biochemically distinguishable from Murraya paniculata establishing its specific chemical profiles along with correlating it to multi-endpoint pharmacological profiling. Collectively, these findings highlight the plant's considerable pharmaceutical potential and provide methodological and mechanistic insights that move the field beyond previous fragmented reports

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