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Research Article

Phytochemical Profiling and Evaluation of the Hepatoprotective Potential of Aglaia Simplicifolia

Nasiya N.[1*], Dr. Abhinandan Danodia[2], Dr. Kavitha K. V.[3]

[1] *Research Scholar, Department of Pharmacy, Shri JJT University, Jhunjhunu, Rajasthan, India.*
[2] *Department of Pharmacy, Shri JJT University, Jhunjhunu, Rajasthan, India.*
[3] *Professor and Head, Department of Pharmacology, Jamia Salafiya Pharmacy College, Pulikkal, Malappuram District, Kerala, India.*

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ABSTRACT

Background: *Aglaia simplicifolia*, a species belonging to the family Meliaceae, is traditionally used in ethnomedicinal practices for various ailments. However, its hepatoprotective potential has not been comprehensively validated scientifically. Objective: This study aimed to evaluate the pharmacognostic characteristics, preliminary phytochemical composition, and in vitro hepatoprotective activity of the ethanolic leaf extract of *A. simplicifolia* against chemically induced hepatic injury. Methods: Dried leaves of A. simplicifolia were extracted with 95% ethanol using a Soxhlet apparatus. Pharmacognostic evaluation and Preliminary phytochemical screening was performed using standard qualitative tests. Hepatoprotective activity was evaluated in an isolated primary rat hepatocyte model. The cells were exposed to four hepatotoxic agents: diethylnitrosamine (DEN), cisplatin, thioacetamide (TAA), and rifampicin (RMP). Cell viability was determined using the Trypan blue exclusion method, with silymarin (100 µg/mL) as the reference standard. Results: In the evaluation we measured physicochemical parameters. Preliminary phytochemical screening showed that the extract contains alkaloids, flavonoids, steroids, glycosides, saponins, tannins and carbohydrates. In the hepatocyte cell viability assay I observed that the extract at 100 µg/mL improved cell viability in all four models. Cell viability was 73.09% for DEN 72.56% for cisplatin, 78.31% for TAA and 84.77% for RMP. For comparison silymarin produced cell viability of 81.14%, 78.31%, 83.06% and 84.60% in the models. In the rifampicin-induced hepatotoxicity model the extract gave a higher cell viability of 84.77%, than silymarin's 84.60%. Conclusion: The ethanolic leaf extract of A. simplicifolia showed in vitro hepatoprotective activities. These preliminary findings suggest that the extract may contain constituents with biological

***Corresponding Author:** Nasiya N.
Address: Research Scholar, Department of Pharmacy, Shri JJT University, Jhunjhunu, Rajasthan, India
Email ✉: nasiyaluqman@gmail.com

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activity relevant to hepatic protection.Further studies, including bioassay-guided isolation, characterization of active constituents, and in vivo evaluation, are required to establish its efficacy and safety.

INTRODUCTION

Higher plants have historically been important sources of drugs and lead compounds for natural-product-based drug discovery. Interest in plant species used in traditional and herbal medical systems remains high because they may provide compounds with useful biological activities.[1] The family Meliaceae, which is widely distributed in tropical and subtropical regions, is particularly important in this regard. Plants belonging to this family have traditionally been used to manage inflammatory disorders, infections, and various other ailments. Phytochemical investigations of different plant parts, including roots, stems, bark, twigs, leaves, and fruits, have identified approximately 326 structurally diverse compounds, including alkaloids, limonoids, triterpenoids, and nitrogen-containing flavaglines.[2]

Within the family Meliaceae,[2] *Aglaia*[3,4] is one of the largest genera, comprising approximately 120–150 species distributed predominantly in the tropical rainforests of Southeast Asia and Australia.[3,4] Species of *Aglaia* are generally medium-sized trees, reaching up to 6 m in height, with greyish-brown, smooth bark and a reddish-brown blaze. Young branches are covered with stellate hairs and may exude a small amount of white latex. The leaves are simple, alternate, and spirally arranged, with petioles measuring approximately 1.3–4 cm in length. The leaf lamina is elliptic to obovate, measuring about 9.5–22.5 × 4.9–10.4 cm, with an acuminate apex, an obtuse or acute base, entire margins, prominent secondary veins on the lower surface, and reticulate tertiary venation.[5]

Phytochemical studies have revealed that *Aglaia* species are rich in secondary metabolites, among which flavaglines are one of the most characteristic classes of natural products.[2–6] Flavaglines are highly bioactive flavolignans formed through the cycloaddition of a flavonoid nucleus with a cinnamic acid-derived moiety. They have attracted considerable attention because of their diverse pharmacological activities.[6,7]

Aglaia simplicifolia has been reported in traditional medicine for the treatment of tumors, skin diseases, fever, diarrhea, and dysentery, and for uses associated with astringent and antipyretic remedies.[5] However, despite its ethnomedicinal importance, scientific validation of its biological activities remains limited. Studies on other *Aglaia* species, including *A. argentea, A. oligophylla, A. elaeagnoidea, A. spectabilis, and A. cucullata,* have reported antifungal activity of flavaglines against phytopathogenic fungi such as Botrytis cinerea and Pestalotiopsis species.[6,8] These findings relate to other species of the genus and should not be taken as direct evidence of the activity of *A. simplicifolia. Aglaia* species have also been reported to possess anti-inflammatory, cytotoxic, antimicrobial, and other biological activities.[4,9]

A research gap therefore remains regarding the pharmacological activities of *A. simplicifolia*. Although the genus *Aglaia* is recognized as a source of flavaglines and other secondary metabolites with diverse biological properties, published evidence specifically addressing the hepatoprotective activity of *A. simplicifolia* appears limited.[2–9] Therefore, the present study aimed to investigate the phytochemical profile, in vitro hepatoprotective potential of the ethanolic leaf extract of *A. simplicifolia.*

MATERIALS AND METHODS

MATERIALS

All chemicals and solvents used in the studies preliminary phytochemical screening and in-vitro hepatoprotective studies were of analytical grade. Ethanol was used for the preparation of the plant extract. Trypsin (0.25%) and Hanks Balanced Salt Solution (HBSS) were used for hepatocyte isolation and washing respectively. Trypan Blue was used to assess hepatocyte viability by the dye exclusion method. Methanol was used as a solvent, for sample preparation wherever required.

2.2. Collection and authentication of the plant

Leaves of *Aglaia simplicifolia* were collected from a local area in Wayanad, Kerala, India. The plant material was authenticated by Dr. Pradeep, Botanist, University of Calicut, Kerala, India.

2.3. Pharmacognostical studies

2.3.1. Preparation of the extract of Aglaia simplicifolia

The dried, coarsely powdered leaves of Aglaia simplicifolia (500 g) were packed in a Soxhlet apparatus and extracted continuously using 450 mL of 95% (v/v) ethanol. The extract was concentrated under reduced pressure at a controlled temperature and dried in a desiccator. The dried extract weighed 75 g, corresponding to a yield of 15% (w/w). The extract was used for phytochemical investigation and pharmacological studies.[10]

2.3.2. Preliminary phytochemical screening

Preliminary phytochemical screening was performed on the ethanolic extract of *Aglaia simplicifolia.* Standard qualitative tests were used to detect different classes of metabolites: Mayer's, Hager's, and Dragendorff's reagents for alkaloids; Salkowski's and Liebermann–Burchard reactions for steroids and triterpenoids; Keller–Killiani, Baljet's, Legal's, and Bornträger's tests for glycosides; the foam test for saponins; Molisch's, Barfoed's, Fehling's, and Benedict's tests for carbohydrates; ferric chloride and Shinoda tests for phenolic compounds and flavonoids, respectively; the ferric chloride test for tannins; and Millon's, xanthoproteic, Biuret, and ninhydrin tests for proteins.[10,11]

2.3.3. Physicochemical parameters

The shade-dried plant material was evaluated for foreign matter, moisture content, total ash, acid-insoluble ash, ethanol-soluble extractive value, and water-soluble extractive value using standard procedures.

2.4. Biological activity

2.4.1. In vitro hepatoprotective activity

Liver tissue was collected from Wistar albino rats in a beaker containing Hank's balanced salt solution (HBSS) and cut into small pieces using sterile scissors. The minced tissue was washed 2–3 times with HBSS to remove red blood cells until the supernatant became clear. Approximately 1 g of processed tissue was treated with 20 mL of pre-warmed 0.25% trypsin solution in HBSS at 37 °C and gently stirred for 10 min. The suspension was allowed to settle for 20–30 min, after which the supernatant was decanted and discarded. Trypsinisation was continued for 15 min with an equal volume of fresh trypsin solution. Large tissue fragments were allowed to settle, and the supernatant was passed through a 230-mesh nylon filter. The remaining pellet was subjected to trypsinisation 2–3 times until the tissue was dissociated. The resulting cell suspension was centrifuged at 2000 rpm for 3 min, and the obtained cell pellet was washed 2–3 times with DMEM, with centrifugation repeated after each washing step. The cells were resuspended in complete medium and incubated at 36.5 °C for 1.5 h to allow recovery.

The in-vitro hepatoprotective activity of the ethanolic leaf extract of *Aglaia simplicifolia* (AS) was evaluated against four hepatotoxic agents, namely diethylnitrosamine (DEN), cisplatin, thioacetamide (TAA), and rifampicin (RMP), using the Trypan blue exclusion method. The viable hepatocyte suspension was divided into four treatment groups for each hepatotoxic agent, with six replicates per group (n = 6). For the DEN-induced hepatotoxicity model, Group I served as the untreated control, Group II received DEN (200 µg/mL) as the toxicant control, Group III received silymarin (100 µg/mL) along with DEN (200 µg/mL) as the standard treatment, and Group IV received AS extract (100 µg/mL) along with DEN (200 µg/mL) as the test treatment. Similarly, in the cisplatin-induced hepatotoxicity model, Group I served as the untreated control, Group II received cisplatin (8 µg/mL), Group III received silymarin (100 µg/mL) along with cisplatin (8 µg/mL), and Group IV received AS extract (100 µg/mL) along with cisplatin (8 µg/mL). In the TAA-induced hepatotoxicity model, Group I served as the untreated control, Group II received TAA (100 µg/mL), Group III received silymarin (100 µg/mL) along with TAA (100 µg/mL), and Group IV received AS extract (100 µg/mL) along with TAA (100 µg/mL). Likewise, in the rifampicin-induced hepatotoxicity model, Group I served as the untreated control, Group II received rifampicin (100 µg/mL), Group III received silymarin (100 µg/mL) along with rifampicin (100 µg/mL), and Group IV received AS extract (100 µg/mL) along with rifampicin (100 µg/mL).

2.5. Statistical analysis

Data were analysed using Graph Pad Prism version 9.0 and are presented as the mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple-comparisons test.

RESULTS

3.1. Pharmacognostical studies

3.1.1. Physicochemical properties

Table 1 presents the physicochemical properties of *Aglaia simplicifolia* leaves. The measured values are reported in

Table 1: Physicochemical Properties and Extractive Values of Aglaia Simplicifolia Leaves

S. No	Name of the plant and part used	Foreign matter	Moisture content	Total ash	Acid insoluble ash	Ethanol soluble extractive value	Water soluble extractive value
1.	Aglaia simplicifolia (leaves)	2%	3%	5-6%	0.85%	1.45%	1.35%

3.1.2. Preliminary phytochemical screening

Preliminary phytochemical screening of the *Aglaia simplicifolia* leaf extract indicated the presence of steroids, alkaloids, flavonoids, glycosides, saponins, tannins, and carbohydrates. The results are presented in Table 2.[10,11]

Table 2: Preliminary Phytochemical Screening of Aglaia Simplicifolia Leaf Extract

Constituents	Test	Aglaia simplicifolia
Carbohydrates	Molisch Test Fehling's Test Benedict's test: Barfoed's test:	+ + + +
Alkaloids	Dragendorff'sTest Wagner'sTest Mayer'sTest Hager's Test	+ + + +
Steroids and Sterols	Liebermann Burchard test Salkowski test	- +
Glycosides	Legal'stest Baljettest Borntragertest Keller–Killiani test	+ + + +
Saponins	Foam test	+
Flavonoids	Shinoda test	+
Triterpenoids	Noller's test	+

+ Present, - Absent

3.2. Biological activities

3.2.1. In vitro hepatoprotective studies

The hepatoprotective activity of *Aglaia simplicifolia* (AS) was investigated using isolated rat hepatocytes treated with four hepatotoxic agents namely Diethylnitrosamine (DEN), Cisplatin, Thioacetamide (TAA) and Rifampicin (RMP). Silymarin was used as a reference hepatoprotectant.Upon exposure to DEN, cell viability became 32.40%. When cells were treated with silymarin and Aglaia simplicifolia, the cell viability increased to 81.14% and 73.09% respectively. This indicates that the plant extract had a protective effect on hepatocyte cells when treated with DEN, although less protective than silymarin. Upon exposure to Cisplatin, 29.53% of cells were viable. The cell viability increased to 78.31% and 72.56% when cells were treated with silymarin and *Aglaia simplicifolia*.

The plant extract showed a protective effect against hepatocyte damage caused by Cisplatin. Cells treated with TAA showed 31.74% cell viability. When treated with silymarin and Aglaia simplicifolia the cell viability increased to 83.06% and 78.31% respectively. This demonstrated the potential of the plant extract to protect against TAA induced hepatotoxicity. Cells treated with Rifampicin had 34.62% viability. When treated with silymarin and Aglaia simplicifolia the cell viability increased to 84.60% and 84.77% respectively. The plant extract had a very similar cell viability as silymarin with only a small difference of 0.17%.

Overall the hepatocyte cells had increased cell viability when treated with Aglaia simplicifolia extract across all four models. Compared to the other models there was a lower cell viability when compared to silymarin for the DEN, Cisplatin and TAA models. However, the Rifampicin model had similar cell viability values. This suggests Aglaia simplicifolia extract has potential as an in vitro hepatoprotectant.Table 5. In vitro hepatoprotective activity of Aglaia simplicifolia in isolated rat hepatocytes exposed to DEN, cisplatin, thioacetamide, and rifampicin

Table 3: In Vitro Hepatoprotective Activity of Aglaia Simplicifolia in Isolated Rat Hepatocytes Exposed to DEN, Cisplatin, Thioacetamide, and Rifampicin.

Hepatotoxic agent	Group	Treatment	Number of cells counted	Number of viable cells	Percentage viability (%)
DEN	I	Control	105 ± 0.5	90 ± 0.19	85.72
	II	DEN (200µg/mL	105 ± 0.25	34 ± 0.25	32.40
	III	Silymarin(100µg/mL) + DEN	106 ± 0.33	86 ± 0.23**	81.14
	IV	AS(100µg/mL) + DEN	104 ± 0.29	76 ± 0.17**	73.09
Cisplatin	I	Control	106±0.41	89±0.25	83.97
	II	Cisplatin (8µg/mL)	105±0.25	31±0.36	29.53
	III	Silymarin (100µg/mL) +Cisplatin	106±0.37	83±0.19**	78.31
	IV	AS (100µg/mL) +Cisplatin	102±0.17	74±0.21**	72.56
Thioacetamide	I	Control	112 ± 0.71	89 ± 0.22	79.47
	II	TAA (100µg/mL)	104 ± 0.17	33 ± 0.44	31.74
	III	Silymarin (100µg/mL) + TAA	105 ± 0.28	87 ± 0.36**	83.06
	IV	AS (100µg/mL) + TAA	106 ± 0.14	83 ± 0.23**	78.31
Rifampicin	I	Control	102±0.71	91±0.33	89.21
	II	RMP (100µg/mL)	104±0.62	36±0.25	34.62
	III	Silymarin (100µg/mL) + RMP	104±0.15	88±0.27**	84.60
	IV	AS (100µg/mL) + RMP	105±0.26	89±0.13**	84.77

DISCUSSION

This study investigated the in-vitro hepatoprotective potential of the ethanolic leaf extract of *Aglaia simplicifolia* by evaluating its protective effect against different hepatotoxic agents in primary rat hepatocytes. The preliminary phytochemical screening indicated the presence of several classes of constituents, including flavonoids, steroids, alkaloids, glycosides, saponins, tannins, and carbohydrates. These findings are consistent with reports of diverse secondary metabolites in other *Aglaia* species.

The study used hepatocytes isolated from Wistar rats and exposed to four hepatotoxic agents: diethylnitrosamine (DEN), cisplatin, thioacetamide (TAA), and rifampicin (RMP). DEN can undergo metabolic activation and generate reactive metabolites that contribute to cellular injury.[15,16] Cisplatin, TAA, and rifampicin can also cause hepatic cellular damage through different mechanisms. In the present study, exposure to each toxicant reduced hepatocyte viability compared with the respective control groups, whereas treatment with the ethanolic extract of *Aglaia simplicifolia* improved cell viability in all four models. The magnitude of the

protective effect varied across the different hepatotoxicity models.

In the DEN-induced hepatotoxicity model, cell viability increased from 32.40% in the DEN-treated group to 73.09% following treatment with the plant extract, whereas silymarin treatment resulted in 81.14% viability. Similarly, in the cisplatin-induced model, cell viability increased from 29.53% in the toxicant-treated group to 72.56% with the extract, compared with 78.31% with silymarin. In the TAA-induced model, the extract improved cell viability from 31.74% to 78.31%, while silymarin produced 83.06% viability. In the rifampicin-induced model, cell viability increased from 34.62% in the toxicant-treated group to 84.77% following extract treatment, compared with 84.60% with silymarin. These findings indicate that the extract exhibited a protective effect against hepatocyte damage induced by all four toxicants. In the rifampicin model, the viability values obtained with the extract and silymarin were numerically similar.

The observed improvement in hepatocyte viability suggests that the ethanolic leaf extract may contain constituents capable of protecting hepatocytes against different toxic insults. The presence of various phytochemical classes, particularly flavonoids, tannins, and other secondary metabolites, may contribute to the observed protective effect. However, the specific compounds responsible for the hepatoprotective activity were not identified in the present study, and the mechanisms underlying the observed effects remain to be established.

Several limitations should be acknowledged. The study employed an in-vitro primary-hepatocyte model, which cannot fully reproduce whole-organism liver function, systemic metabolism, immune responses, or pharmacokinetic interactions. Cell viability was assessed using the Trypan Blue exclusion method, which primarily reflects cell membrane integrity and does not measure specific liver functions. Overall, the findings provide preliminary in-vitro evidence of the hepatoprotective potential of the ethanolic leaf extract of *Aglaia simplicifolia* against DEN-, cisplatin-, TAA-, and rifampicin-induced hepatocyte toxicity. Further studies are required to establish the underlying mechanisms, efficacy, and safety of the extract before its potential clinical application can be considered.

CONCLUSION

The ethanolic leaf extract of *Aglaia simplicifolia* was associated with improved viability of primary rat hepatocytes exposed to diethylnitrosamine (DEN), cisplatin, thioacetamide (TAA), and rifampicin (RMP). Preliminary phytochemical screening indicated the presence of flavonoids, steroids, alkaloids, glycosides, saponins, tannins, and carbohydrates. The physicochemical parameters evaluated for the plant material included 2% foreign matter, 3% moisture content, 5–6% total ash, and 0.85% acid-insoluble ash.

In the primary rat hepatocyte viability assay, treatment with the ethanolic leaf extract improved cell viability across all four hepatotoxicity models, with viability values ranging from 72.56% to 84.77%. The highest cell viability (84.77%) was observed in the rifampicin-induced hepatotoxicity model, while the lowest (72.56%) was observed in the cisplatin-induced model. These findings provide preliminary in-vitro evidence of the hepatoprotective potential of *Aglaia simplicifolia*.

The study had certain limitations, including its in-vitro design, the use of cell viability as the primary assessment parameter, the absence of additional liver-function biomarkers, the lack of hepatoprotective dose–response studies, and the absence of in-vivo validation. Furthermore, the active phytochemical constituents responsible for the observed protective effects and their underlying molecular mechanisms remain to be identified.

Further studies should include bioassay-guided isolation and characterization of the active constituents using suitable analytical techniques such as LC–MS, GC–MS, and NMR. Additional mechanistic investigations and in-vivo studies evaluating hepatoprotective efficacy and safety are necessary to establish the therapeutic potential of *Aglaia simplicifolia*.

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