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Research Article

THE SECONDARY METABOLITE PROFILING, ANTIOXIDANT, ANTIDIABETIC, ANTIMICROBIAL AND ACUTE ORAL TOXICITY ANALYSIS OF METHANOL EXTRACT OF *ELAEOCARPUS ANGUSTIFOLIUS* BLUME FLOWER

***Ram Darash Pandey*[1, 2], *Susan Joshi*[*1]**
[1]*Central Department of Chemistry, Tribhuvan University, Kirtipur, Kathmandu, Nepal*
[2]*Department of Chemistry, Amrit Campus, Tribhuvan University, Thamel, Kathmandu, Nepal.*
** Corresponding author: susanjoshi68@gmail.com*

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ABSTRACT

In the present research, we investigated the flowers of the plant *Elaeocarpus angustifolius* Blume. This plant is cultivated in the hilly regions of Nepal, primarily for its beads. This is the first attempt to study in Nepal to explore the methanol extract of flower using both qualitative and quantitative analyses. The secondary metabolite screening indicated alkaloids, flavonoids, polyphenols, saponins, glycosides, terpenoids, and reducing sugars in the extract. Several of these metabolites were also confirmed by the GC-MS analysis. The phenolics and flavonoids were quantified by colorimetric tests using the Folin-Ciocalteu reagent (FCR) and $AlCl_3$, respectively. The amount of phenolic and flavonoids in the extract was 169.111 ± 18.867 mg GAE/g at a concentration of 0.644 and 84.401 ± 6.204 mg QE/g at a concentration of 0.582, respectively. The extract was analysed for antioxidant activity by the well-known DPPH method. The IC_{50} value obtained was 70.659 µg/mL, indicating it to be a moderate antioxidant. The extract was also analysed for antidiabetic potential using the α-amylase inhibition assay. The IC_{50} value obtained was 1717.0 µg/mL, indicating its weak antidiabetic action. Antibacterial and antifungal activity effect against tested pathogenic bacteria and fungi were not observed. The flower extract and powder were evaluated for acute oral toxicity in Albino mice by OECD guidelines. They were found to be non-toxic. Due to the high level of phenolic and flavonoid in the extract, this study highlights its potential therapeutic uses for treating diabetes, obesity and antioxidant.

Keywords: Acute oral toxicity, Antidiabetic, Antifungal, Antioxidant.

INTRODUCTION

The research interest in exploring plant-derived bioactive compounds from medicinal plants has been ongoing since the development of human civilisation. Plant synthesis metabolites help them survive in harsh environments. For plants, they serve survival needs, but for humans, they have medicinal value and are useful in treating various diseases (Doss, 2009). Nepal is rich in many medicinal plants that remain largely unexplored or have not been thoroughly studied (Bhattarai *et al*., 2024). One such plant is *Elaeocarpus angustifolius* Blume, popularly known as Rudraksha. It is mainly found in the Himalayan and mountainous regions of Nepal. The genus *Elaeocarpus* comprises nearly 360 species, mainly found in Asian countries including Nepal, India, Thailand, and Bhutan (Joshi *et al*., 2020). According to literature reviews, it is evident that Ayurvedic and folk medicine mention various uses, such as treating liver issues, migraines, asthma, arthritis, stress, depression, and anxiety. Currently, research has focused mainly on the leaves and fruits of this plant. There are many descriptions of traditional applications of the plant in reviews. To mention a few: to treat smallpox and chickenpox, promote sound sleep, neutralise venomous insect bites, enhance memory, treat coma and hysteria, and alleviate symptoms of brain fever (Gupta *et al*., 2016). The antibacterial activity of dried fruit extracts of *E. sphaericus* in various solvents has been analysed. The results demonstrated dopaminergic and serotonergic action of the 50% ethanol extract of the fruit in rats. Moreover, the results also concluded that the active ingredients in this Ayurvedic formulation may have antidepressant efficacy (Bopaiah *et al*., 2000). The bark extract in chloroform displayed significant anticancer activity against human cancer cell lines (Ito *et al*., 2002). The fruit extract in 90% ethanol from *E. ganitrus* demonstrated antihypertensive effects in experimental models of acute hypertension induced by nicotine and adrenaline (Singh *et al*., 2010). The fruit of *E. sphaericus* exerts analgesic effects when administered at a dose of 100 mg/kg body weight.

Ethanolic extracts of *E. ganitrus* leaves have been tested for antioxidant capacity, including reducing power, metal chelation, 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging, and hydroxyl radical scavenging activities (Kumar *et al*., 2014). Aqueous extracts from *E. ganitrus* seeds have shown antihypertensive effects in rats with hypertension and renal artery occlusion, with encouraging results (Indriatie *et al*., 2020). The methanolic extract of *E. sphaericus* fruit exhibits anxiolytic effects at a dose of 200 mg/kg body weight (Shah *et al*., 2010). The antifungal assay showed significant activity against *Candida albicans*, with ethanol and chloroform extracts among the tested solvents. Different solvent extracts, like chloroform, ethanol, and water, demonstrated inhibition of *Aspergillus niger* (Singh *et al*., 2010). Fruit ethanol extracts of *E. ganitrus* have been studied for sedative, hypnotic, tranquillising, anticonvulsant, antiepileptic, and antihypertensive effects at different doses (Singh *et al*., 2010). *E. ganitrus* bark extract in chloroform demonstrated notable anti-inflammatory effects compared to synthetic non-steroidal diclofenac sodium (Lakshmi *et al*., 2016). The leaf extract of *E. sphaericus*, derived from chitosan, demonstrated hypoglycaemic effects on normal rats (Kumar *et al*., 2014). The fruit of *E. ganitrus* showed a significant hepatoprotective effect on mice exposed to carbon tetrachloride (Srikanth *et al*., 2018). Both aqueous and hydro-methanolic extracts of *E. sphaericus* leaves contained phenolic and flavonoids, with the hydro-methanolic extract displaying substantial antibacterial and antioxidant activities (Sharma *et al*., 2018). Additionally, the bark extracts of *E. sphaericus* in different solvents, like aqueous, methanolic, and chloroform, displayed thrombolytic and antioxidant properties. The ethyl acetate extract from *E. ganitrus* bark demonstrated dose-dependent inhibition of α-amylase *in vitro* (Talukdar *et al*., 2017). The water-methanol extract of *E. ganitrus* fruit exerted anxiolytic effects in mice (Rauniar *et al*., 2012). The leaf extract was evaluated for its antioxidant potential with promising results (Kumar *et al*., 2008). Hong *et al*. (2019) reported nine new alkaloids and five known compounds from branches and leaves of *E. angustifolius*. A comprehensive review on this plant by Banu *et al*. (2024) mainly mentions the studies related to leaves, bark, seeds and fruits. A recent study by Gopal *et al*. (2025) focused on an *in silico* antidiabetic study on the seed of this plant. Thus, previous studies on *Elaeocarpus* have mostly focused on the plant's leaves, bark, seed and fruit. To our knowledge, there has been no published research on the phytochemistry of the flower extract and its biological applications on this plant. Moreover, there is no isolation reported from the flower of this plant. Therefore, our investigation chiefly focuses on analysing the secondary metabolites in different solvent extracts of the flower and conducting bioassays of one of the flower extracts to get a futuristic idea on isolating bioactive compounds.

MATERIALS AND METHODS

Plant Material and Extraction

Elaeocarpus angustifolius Blume flowers were collected from Sanobharyang (27°43'14.3"N 85°17'17.5" E), Kathmandu. It was taxonomically identified by the National Herbarium and Plant Laboratory, Godavari, under voucher code RDP-01. The flowers were dried in the shade for 15 days. The flowers were ground into powder using an electric grinder and then macerated in hexane, dichloromethane, ethyl acetate, methanol, and water, respectively, in parallel, to obtain different solvent extracts (powder-solvent ratio 1:4 w/v) over three days with intermittent shaking. It was then filtered through cotton. To concentrate it, a rotary evaporator set was used at 40°C and 120 rpm to produce crude extracts, which were subsequently stored in vials at 4°C for further analysis.

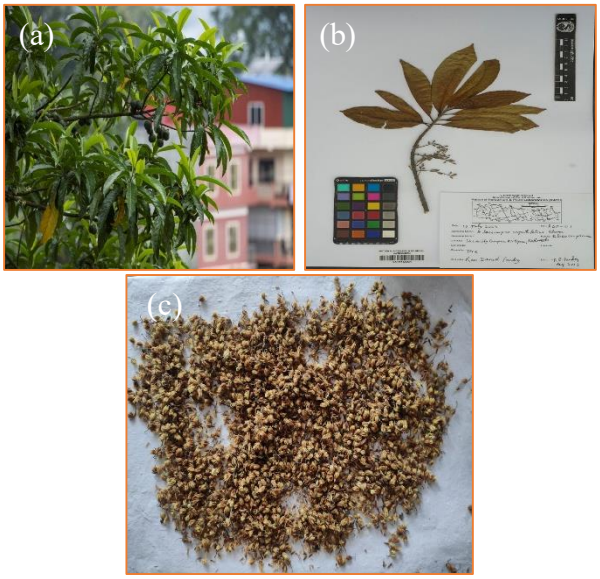

Figure 1. (a) *Elaeocarpus angustifolius* Blume plant (b) Herbarium of plant leaf (c) flower of the plant.

Phytochemical Screening

The *Elaeocarpus angustifolius* Blume flower crude extracts in different solvents were tested for secondary

metabolites using a standard protocol (Harborne, 1984; Joshi *et al*., 2021).

FTIR Analysis of Crude Extract

The crude methanol flower extract was analysed using FTIR (PerkinElmer Spectrum 2 IR Version 10.6.2) within the range of 4000 to 400 cm^{-1}. The functional groupings were deduced using spectral data.

Ultraviolet-Visible Spectroscopy of Crude Extract

The UV-vis spectrum of the methanol flower extract was recorded in the range of 200-800 nm, using methanol as the blank, with a double-beam spectrophotometer (Labtronics, 2802). A 20-ppm clear solution of the plant extract was prepared in methanol for the analysis. The electronic transitions were identified from the spectral data.

Analysis by GC-MS Spectrometry

The GC/MS spectrometry analysis of the flower methanol extract was performed by utilising a GC-MS-QP2010 (Shimadzu) having an AOC-20i auto sampler for metabolites extraction. The separation was conducted using an Rtx-5MS capillary. The following setup was used for the column: dimension (30 m × 0.25 mm × 0.25 µm), carrier gas (helium), and pressure of 500-900 kPa; the oven temperature 60°C, the ion source temperature 200°C, the interface temperature, and the injection temperature 280°C. The splitless injection mode was employed. The pressure measured was 80 kPa, and the purge flow rate was 3.0 mL/min. The MS acquisition mode was configured for normal scanning at a speed of 2500, with a duration of 14.67 minutes and a mass (m/z) scan range of 40-500.

Total Phenolic Contents (TPC) Determination

For this analysis, the protocol described by Chandra *et al*., (2014) was followed. For this test, 20 µL extract (1 mg mL^{-1}) was taken, followed by 100 µL of FCR (1:10 dilution in DW) in a 96-well plate. After 15 minutes, the initial absorbance was recorded at 765 nm. About 80 µL of sodium carbonate (7.5% in distilled water) was added to the well and kept in the dark for 30 minutes. The final absorbance was measured at 765 nm with a microplate reader. In this analysis, gallic acid was used as a standard. The result of this analysis is generally reported as mg GAE per gram. The experiments were conducted three times. For the calculation of the TPC, the following formula was used:

$$TPC = C \times V/m \quad \ldots (1),$$

Where, V = the volume of the extract in mL,

m = mass of extract in grams,

and C = gallic acid concentration (mg/mL).

Total Flavonoid Contents (TFC) Determination

For the analysis, the protocols mentioned by Javanmardi *et al*., (2003) were followed. A 130 µL mixture (20 µL extract + 110 µL distilled water) was taken in triplicate in a 96-well plate. The initial absorbance of the mixture at 415 nm was recorded. After this, the following solutions were added to each well sequentially: 5 µL of $AlCl_3$ (10 % solution in methanol), 5 µL of potassium acetate, and 60 µL of ethanol. After 30 minutes, the final absorbance of the mixture was recorded at 415 nm. The result of this analysis is generally reported as mg quercetin equivalent (QE/g). The experiments were performed in triplicate. For the calculation of the TFC, the following formula was used:

$$TFC = C \times V/m \ldots (2),$$

Where V = the extract volume in mL,

m = mass of extract in grams

and C is the quercetin concentration in milligrams per millilitre.

Antioxidant Assay

The *in vitro* antioxidant assay of flower extract was performed by using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay as by described by Škerget *et al*. (2005). Solutions of plant extract in different concentrations were prepared. To 50 µL of plant extract at different concentrations, 150 µL of DPPH solution (0.1 mM DPPH solution in methanol) was added in triplicate, in a 96-well plate. The plate was left in the dark for 30 minutes before the absorbance measurement at 520 nm on a microplate reader. The antioxidant power was compared with ascorbic acid standard. The scavenging activity percentage was determined using the equation:

$$\text{Scavenging percentage} = [(X_1 - X_2) / X_1] \times 100 \quad \ldots (3),$$

Where X_1 = absorbance of the control solution, and X_2 = absorbance of the sample or standard. Experiments were performed in triplicate.

Antibacterial Assay

The antibacterial assay was performed using the well diffusion method, as described in the standard protocol (CLSI, 2012). The standard bacterial cultures utilised in this investigation were *S. aureus* (ATCC 25293), *S. typhi* (ATCC 14028), *E. coli* (ATCC 25922), and *K. pneumoniae* (ATCC 13883). A sample concentration of 50 mg/mL was used in this study. Ampicillin (1 mg/mL) was used as a positive control, and dimethyl sulfoxide (DMSO) was used as a negative control.

Antifungal Assay

The antifungal assay was conducted similarly to the antibacterial assay in accordance with the CLSI (2012) guidelines. Two types of fungi used in this study were *Alternaria* and *Fusarium* spp. (provided by Nepal Agriculture Research Council, NARC). The test organism was cultured initially in potato dextrose broth (PDB). Then, 100 µL of inoculum was spread onto Mueller-Hinton Agar (MHA) plates and 20 µL of samples of concentration (50 mg/mL) were introduced into the wells. The plates were incubated at 28°C for one week. The positive control was cycloheximide (50 mg/mL), and the negative control was DMSO. The zone of inhibition (ZOI) was measured.

***In vivo* Acute Oral Toxicity Test (AOTC)**

Flower extract and powder were tested for its toxicity on mice *in vivo* at the Department of Plant Resource (DPR) laboratory, Thapathali, Kathmandu. AOTC for flower extract and powder was performed on Albino mice in accordance with the guidelines of OECD TG 425 Up and Down Procedure (OECD, 2002). In this experiment, we administered a single dose of the flower extract and powder to three female Albino mice based on its body weight. They were monitored for 14 days, especially during the first 4 hours and up to 24 hours afterwards. The death of any of the three mice within 14 days indicates the toxicity of the extract or powder. If no death, the extract or powder was considered non-toxic. If toxicity was observed, further tests with reduced doses were applied to validate the results. Toxicity is expressed as the LD_{50} value.

Antidiabetic Assay

The flower extract was used for an antidiabetic assay following a modified protocol described elsewhere (Kim *et al*., 2005). A working solution of α-amylase (0.5 mg in 1 mL of phosphate buffer at pH 6.9) was prepared. The plant extract (1 mg/mL) in methanol was utilised for the study. The methanol-dissolved extract was further diluted in phosphate buffer to produce solutions of various concentrations (100, 75, 50, and 25) µg/mL. A 200 µL aliquot of α-amylase was combined with 200 µL of each extract to incubate for 10 minutes at 30°C. Subsequently, a 200 µL starch solution (1% weight/volume in water) was added to all tubes and incubated for a further 3 minutes. The reaction was quenched by adding 3, 5-dinitrosalicylic acid.

The positive control involved the substitution of plant extract with 1 mg/mL metformin in the reaction mixture. The tube representing 100% enzyme activity contained no plant extract. All tubes were then incubated at 85°C for 10 minutes. After incubation, 5 mL of distilled water was added to each tube, and the optical density was measured at 540 nm. To calculate the inhibition rate, we used the formula:

Inhibition rate =

$$\frac{\text{Abs of 100 \% enzyme activity} - \text{Abs of sample reaction}}{\text{Abs of 100 \% enzyme activity}} \times 100 \quad \ldots.(4)$$

Here, Abs stands for absorbance.

RESULTS AND DISCUSSION

Phytochemical Screening

The examination of phytoconstituents shows that all extracts lack volatile oils but contain alkaloids, terpenoids, flavonoids, polyphenols, saponins, glycosides, and reducing sugars, as illustrated in Table 1. The qualitative analysis provides an idea of the metabolites in the crude extract. The additional analysis further supports the presence of such metabolites. Consequently, UV, IR, and GC-MS analyses were conducted to gather more information.

Table 1: Test for different phytoconstituents.

S.N.	Phytochemical constituents	Hexane extract	DCM extract	EtOAc extract	MeOH extract	Aqueous extract
1	Volatile oil	-	-	-	-	-
2	Alkaloids	-	-	+	+	-
3	Terpenoids	+	+	+	+	+
4	Flavonoids test	+	+	+	+	+
5	Coumarins	-	+	+	++	+
6	Quinones	+	+	+		
7	Polyphenols	-	-	-	++	++
8	Saponin	-	-	-	-	+
9	Tannin	-	-	-	-	-
10	Glycosides	+		+	++	++
11	Reducing Sugar	+	+	+	+	+

Note: + means present, ++ means very good, - means absent

UV-visible and FTIR Spectroscopy Analysis

The UV-visible spectra, as shown in Figure 2(a), reveal the presence of many bonds, conjugated systems, and heteroatoms with lone pairs, and chromophoric groups in the secondary metabolite (Gautam *et al*., 2025). Furthermore, for colourless chemicals, the absorption maxima occur within the 200-400 nm range, but for colourful compounds, including chlorophyll, anthocyanin, carotenoids, anthraquinones, and flavonoids, they are found in the 400-800 nm range at various wavelengths (Harborne, 1984). The peaks at 391 nm, 496 nm, and 700 nm are due to $\pi \rightarrow \pi^*$ transition in a highly conjugated system. The peaks at 735 nm and 739 nm are due to chlorophyll. The peaks at 749 nm, 753 nm, and 758 nm may be due to $\pi \rightarrow \pi^*$ transition in a highly conjugated macrocyclic chromophore. The peak at 790 nm may be due to aggregated chlorophyll derivatives.

The FTIR peaks, as shown in Figure 2(b), are observed at wavenumbers 3347 cm^{-1} (-OH stretching), 2924 cm^{-1} (asymmetric C-H stretching), 2846 cm^{-1} (symmetric C-H stretching), 1712 cm^{-1} (C=O stretching), 1607 cm^{-1} (C=C stretching), 1446 cm^{-1}, 1340 cm^{-1}, 1203 cm^{-1} (C-C stretching), and 1019 cm^{-1} (C-O stretching). The FTIR peaks signify the functional groups contained in the secondary metabolite (Silverstein *et al*., 2014). Thus, the FTIR analysis clearly indicates the presence of functional groups like -OH, >CO, and C=C bond in the secondary metabolite present in the extract.

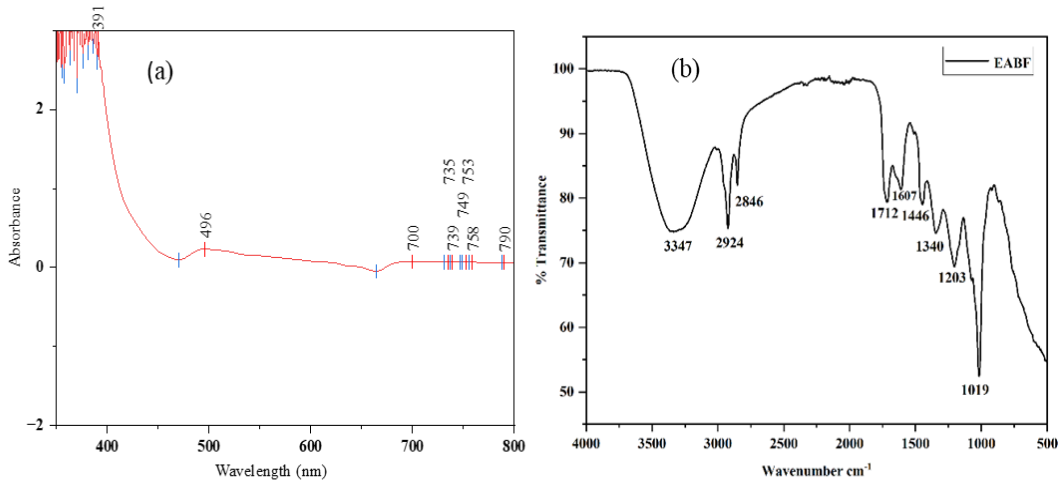

Figure 2: (a) UV-vis spectrum, & (b) FTIR spectrum.

Analysis by GC-MS

The GC-MS of the flower extract identified 22 compounds based on the comparison with the NIST library database with the instrument. The main ones are benzoic acid, 9, 12-octadecadienoic acid (Z, Z), Lup-20(29)-en-3-one, thymol, and 6, 9, 12, 15-docosatetraenoic acid, as depicted in Table 2 and chromatogram in Figure 3. The structures of some bioactive metabolites are shown in Figure 7.

The metabolites listed mainly consist of acids, triterpenoids, monoterpenoid phenols, apocarotenoids, coumarin derivatives, stilbenoids, and vitamin E derivatives. This GC-MS analysis data provides evidence of known compounds in the flower extract with a NIST library match. The functional group peaks, showing the presence of carboxyl group, hydroxyl group and double bonds in the metabolite in the FTIR spectrum of the extract, further support this finding.

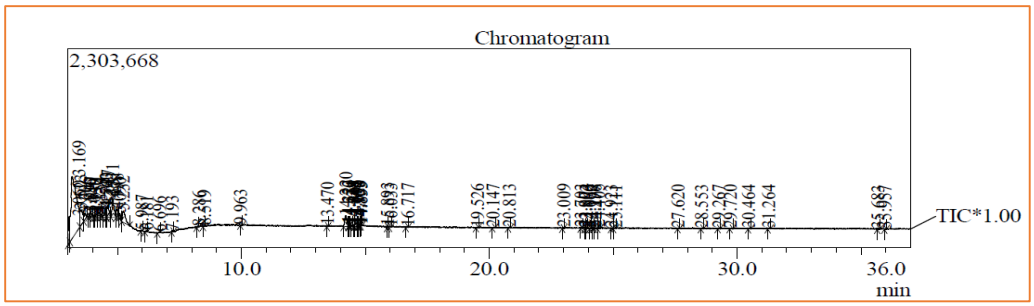

Figure 3. GC-MS chromatogram of the flower methanol extract.

Table 2. GC-MS analysis of flower methanol extract.

Peak	Retention time	Area %	Base m/z	Name
4	3.706	1.67	105.05	Benzoic acid
17	5.232	5.09	55.05	9,12-Octadecadienoic acid (Z,Z)-
19	6.181	0.22	55.05	7-Hydroxy-6,9a-dimethyl-3-methylene-decahydronaphthalene
22	8.286	0.21	281.05	6,10′-Dioxo-11′-oxaspiro[cyclohexane-1,8′-tricyclo[4.3.1.0²,⁷]decan]-3′-yl-2,5,7,8-tetramethylchroman (α-Tocospiro A)
23	8.519	0.16	73.00	2-Hydroxybenzeneacetic acid
26	14.220	3.75	55.05	Lup-20(29)-en-3-one (triterpenoid)
28	14.350	0.15	207.00	Thymol (monoterpenoid phenol)
29	14.474	0.24	208.00	2,6-Dihydroxybenzoic acid
30	14.543	0.15	209.00	3-Hydroxy-.beta.-damascone (apocarotenoids)
31	14.605	0.80	209.00	6,9,12,15-Docosatetraenoic acid
32	14.693	0.28	73.00	Thymol
33	14.720	0.21	133.10	3-(3,4-Dimethoxycinnamoyl)-4-hydroxy-6-methylchromen-2-one
34	14.758	0.15	73.05	Homovanillic acid
37	15.893	0.18	73.00	2'-Deoxyadenosine
38	16.033	0.22	281.05	5-Methylsalicylic acid
40	19.526	0.15	97.00	Pinosylvin (stilbenoids)
46	23.903	0.18	207.00	Silicic acid
52	25.111	0.20	207.00	Thymol
53	27.620	0.17	73.05	D-Erythro-Hex-2-enonic acid
58	31.264	0.21	207.00	Thymol
59	35.683	0.23	73.05	Salicyl alcohol
60	35.957	0.18	73.05	Beta.-d-Arabinofuranosyl

Toxicity Test

The methanol flower extract and powdered forms were tested for acute oral toxicity on Albino mice fed a single dose. No fatalities were recorded in any of the mice at this dose level throughout the 14 days observation period, and the LD_{50} values for the flower methanol extract and flower powder are included in Table 3. According to OECD criteria, the LD_{50} indicates that both the flower extract and the original flower powder are safe for human use. Consequently, the flower extract is essential for the advancement of innovative biological applications.

Table 3. LD_{50} values for the flower powder and extrac

Sample	LD_{50} value
Flower powder of *Elaeocarpus angustifolius* Blume	>2000 mg/kg BW
Flower extract of *Elaeocarpus angustifolius* Blume	>2000 mg/kg BW

Estimation of Phenolic Content

The TPC value of the flower extract was 169.111 ± 18.867 mg GAE/g, whereas these values for hexane, dichloromethane, ethyl acetate, methanol, and aqueous extracts were 0.241, 8.256, 66.790, 92.504, and 57.12 mg GAE/g, respectively. The methanol extract showed the greatest TPC value, while the hexane extract demonstrated the lowest. Figure 4 also signifies that the TPC value progressively rises with an increase in solvent polarity. The polar solvent is more effective in extracting phenolic compounds due to the presence of many polar -OH groups. The phenolic present in the flower extract can exert antioxidant properties due to the presence of -OH groups that act as a free radical scavenger (Dai & Mumper, 2010). These free radicals are notorious and cause several disorders in the body, including the well-known ageing phenomenon. Therefore, we performed total phenolic content analysis of the flower extract in this study.

Estimation of Flavonoid Content

The amount of flavonoid in the extract was 84.401 ± 6.204 mg QE g^{-1}. The TFC values for several solvent extracts-hexane, dichloromethane, ethyl acetate, methanol, and aqueous were determined to be 8.173, 10.02, 9.26, 50.86, and 16.58 mg QE/g, respectively. The CH_3OH extract showed the highest TFC value, whereas the hexane extract displayed the lowest. This is the inaugural comprehensive TFC investigation (Figure 5) on methanol and various other solvents.

The flavonoids present in plants exert antioxidant activity due to the presence of many –OH groups, which act as free radical scavengers (Yang *et al*., 2025). Moreover, flavonoids exert antidiabetic activity by several mechanistic pathways, as described in Figure 8.

DPPH Assay

The maximum inhibition percentage was 77.52 at 100 µg/mL, whereas the minimum % was 41.51 at 60 µg/mL. The IC_{50} value for the methanolic extract of the flower was assessed from the calibration curve of standard ascorbic acid (Figure 6), utilising GraphPad Prism. The measured value was 70.65 µg/mL in contrast to the normal ascorbic acid concentration of 25.74 µg/mL.

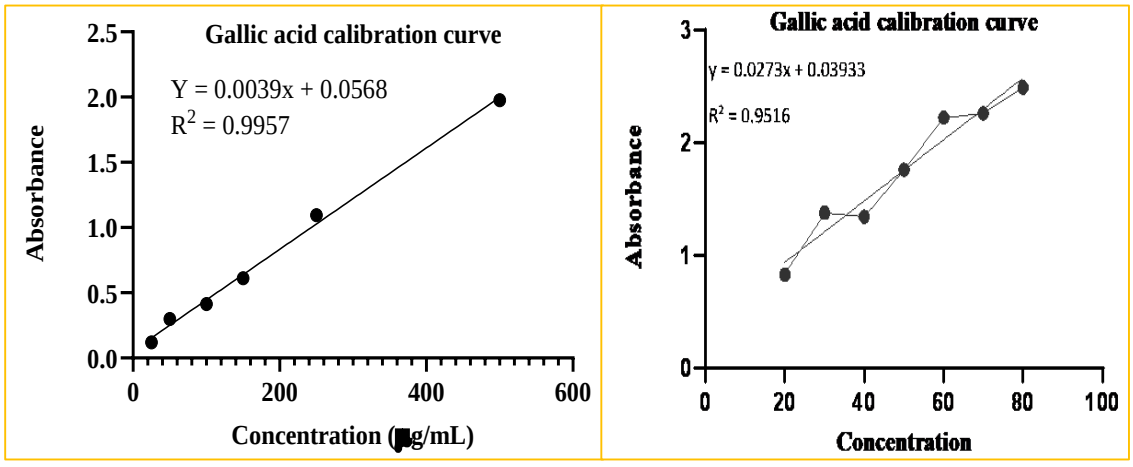

Figure 4. a) Standard calibration curve for methanol extract, b) Standard calibration curve for different solvent extracts.

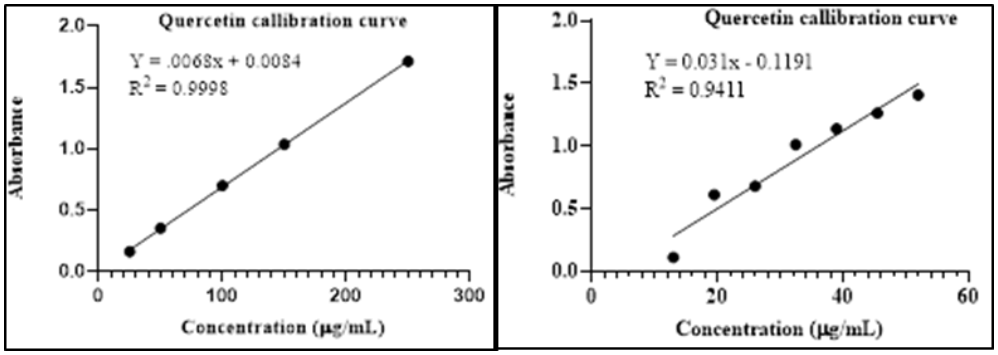

Figure 5: Standard calibration curve for a) methanol extract, b) different solvent extracts.

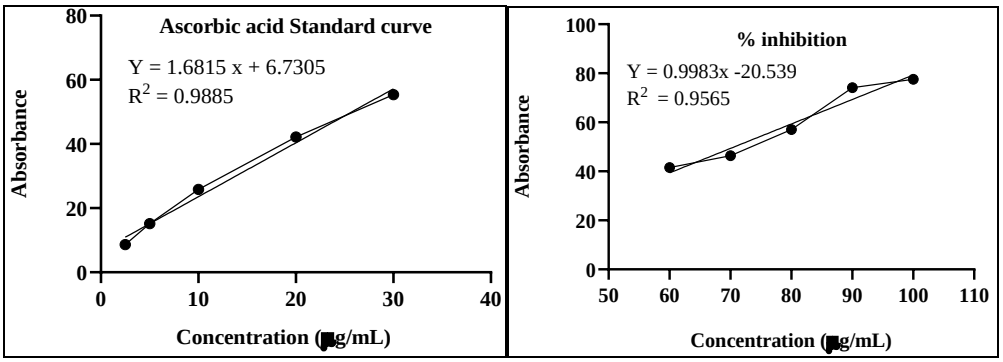

Figure 6. a) Ascorbic acid standard calibration curve, b) Concentration vs percentage inhibition for methanol extract.

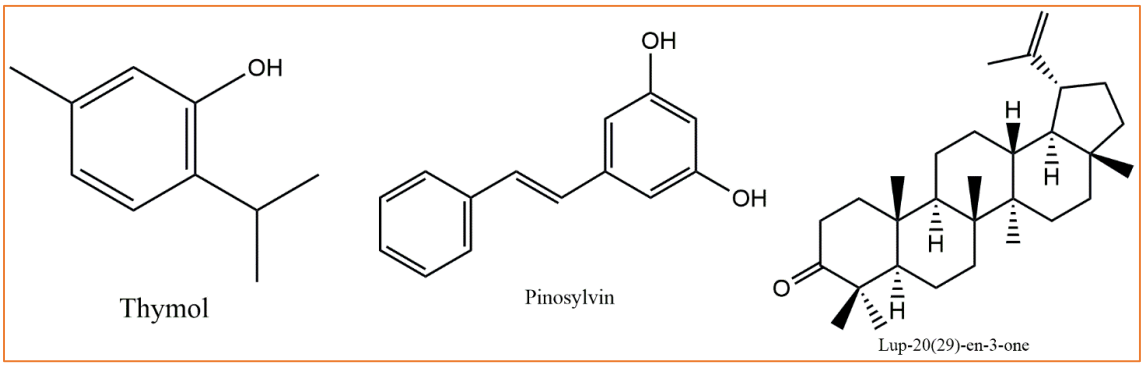

Figure 7: Structures of some bioactive compounds.

The antioxidant activity of the extract mainly results from secondary metabolites such as phenolics and flavonoids. A high content of phenols and flavonoids indicates strong antioxidant and antidiabetic effects. Based on IC_{50} values, a sample's antioxidant property can be classified into five categories: a) IC_{50} <10 µg/mL = very strong antioxidant, b) 10-50 µg/mL = strong antioxidant, c) 50-100 µg/mL = moderate antioxidant, d) 100-250 µg/mL = weak antioxidant, and e) > 250 µg/mL = inactive (Phongpaichit *et al.*, 2007). In our study, the antioxidant activity is moderate compared to the standard ascorbic acid and the ranges specified above. The antioxidant effect may be linked to compounds such as vitamin E, Pinosylvin, Lup-20(29)-en-3-one, and Thymol present in the extract. Thymol is a volatile monoterpenoid known for various biological activities, including antioxidant, antidiabetic, antibacterial against Gram-positive

bacteria, and antifungal against *Candida* and dermatophytes (Escobar *et al*., 2020). Pinosylvin has also been associated with several biological effects, such as potent antibacterial activity against Gram-positive bacteria, antifungal activity against *Candida*, and antidiabetic and anticancer properties (Tshikhudo *et al*., 2025). Lup-20(29)-en-3-one is linked to multiple bioactivities, including antioxidant, antimicrobial, anti-inflammatory, and anticancer effects (Olatunde *et al*., 2025).

Antidiabetic Assay

In the antidiabetic assay, the maximum inhibition percentage observed was 88.54% at a dose of 5000 µg $mL^{-1,}$ while the minimum inhibition percentage observed was 37.20% at a dose of 1000 µg mL^{-1}. The IC_{50} value of the floral extract was determined using the standard calibration curve of metformin with GraphPad Prism, resulting in a value of 1717 µg/mL or 1.717 mg/mL, compared to the standard acarbose (IC_{50} = 0.32 ± 0.16 µg/mL) and metformin (IC_{50} = 19.4 ± 1.4 mM). An IC_{50} value of 36.90 µg/mL was reported in a related earlier study on leaf extract. Metformin is a Biguanide, commonly used first-line drug against type 2 diabetes mellitus. Although the molecular mechanism of action of this drug is not yet clearly understood, it is believed that the insulin sensitivity is improved through modification of the post-receptor signalling pathway (Bösenberg & Van Zyl, 2008).

This finding implies that the leaf extract of the same plant has better antidiabetic properties than the floral extract. Previous research suggests that there is a higher concentration of flavonoids in the leaf compared to the flower extract in our study. The flavonoids present in the flower extract can exert antidiabetic action via different mechanistic pathways, as depicted in Figure 8. It is obvious that flavonoids increase insulin function and β-cell function through the PI3K/AKT pathway, and decrease α-glucosidase and α-amylase by inhibiting GLUT-2. The other mechanistic pathways are vivid in the figure (Ansari *et al*., 2022). The global occurrence of type 2 diabetes is increasing at an alarming rate in people of different age groups (Lin *et al*., 2020). In Nepal, the scenario is not much better, as the diabetes cases are increasing every year (Shrestha *et al*., 2022). Therefore, searching for the plant-based antidiabetic compounds is necessary. Moreover, they are much safer than synthetic ones.

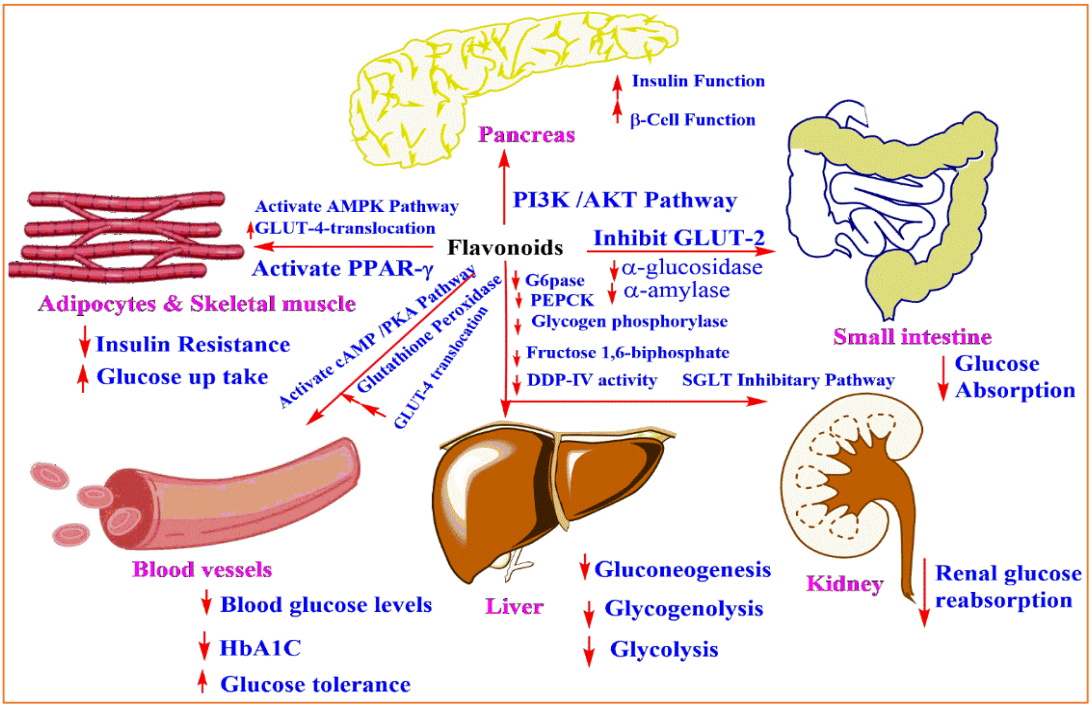

Figure 8. Flavonoids' effects on antidiabetic activity through several mechanistic pathways. (PI3K/AKT = phosphoinositide 3-kinase, GLUT-2 = Glucose transporter-2, GLUT-4 = Glucose transporter-4, AMPK = 5' adenosine monophosphate- activated protein kinase, G6pase = Glucose-6-phosphotase, PEPCK = phosphoenolpyruvate carboxykinase, DDP-IV = Dipeptidyl peptidase-4, SGLT = sodium-glucose linked transporter, PPAR-γ = Peroxisome proliferator–activated receptor-γ, cAMP = cyclic adenosine monophosphate, PKA = protein kinase A, HbA1C = Hemoglobin A1C).

Antibacterial Assay and Antifungal Assay

When the bacteria *B. subtilis* and *S. aureus* were tested using the crude methanolic flower extract of the *E. angustifolius* Blume, the positive control showed a ZOI of 21 mm, while the sample and the negative control showed no inhibition. Except for the positive control, the ZOI was not observed for the sample and the negative control, with the tested bacteria *K. pneumoniae* and *E. coli*. The zones of inhibition for positive control with these bacteria are 22 mm and 16 mm, respectively. The result indicated the lack of antibacterial efficacy against the examined pathogens. Similarly, the ZOI was not observed for the tested fungi, *Alternaria* and *Fusarium* spp., with the methanol extract of the flower. For these fungi, the ZOI observed for the positive control was 21 mm and 20 mm, respectively, indicating the lack of antifungal efficacy of the methanol extract of the flower, in spite of the presence of antimicrobial compounds such as Pinosylvin, Lup-20(29)-en-3-one and Thymol. This may be due to a lower amount of these bioactive compounds or interference of other compounds in the extract. To some extent, this investigation has been supported by previous work on the leaf extract of this plant against *B. subtilis*, *E. coli*, and *K. pneumoniae*, exhibiting no activity; however, *S. aureus* did exhibit a ZOI (Joshi *et al*., 2020). The various studies have reported that the synthetic drugs are gradually becoming resistant to bacteria. Additionally, plant-derived drugs have fewer or no side effects (Matei & Visan, 2025). Therefore, the plant-derived antibacterial drugs are being searched for continuously.

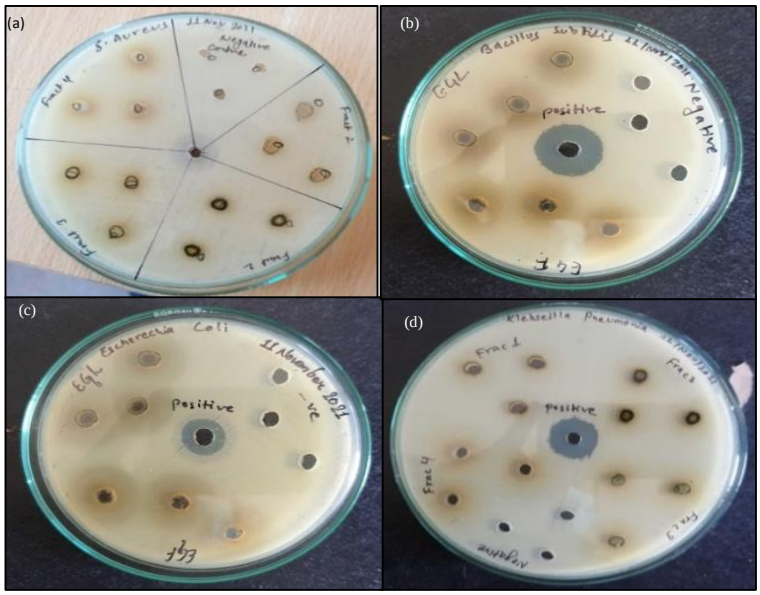

Figure 9: Antimicrobial assay of the flower methanol extract with (a) *S. aureus* (b) *B. subtilis*, (c) E. *coli* and *K. pneumonia.*

CONCLUSIONS

The *E. angustifolius* Blume flower extract and powder were non-toxic in the *in vivo* AOTC study conducted on Albino mice, indicating their suitability for biological uses. The total phenolic content, 169.111 ± 18.867 mg GAE/g, exceeded the total flavonoid content, 84.401 ± 6.204 mg QE/g. The mild antioxidant property and weak antidiabetic properties were observed in comparison to the standard ascorbic acid and metformin, respectively. The significant bioactive substances identified in GC-MS were benzoic acid, Thymol (a monoterpenoid phenol), pinosylvin (a stilbenoid), and Lup-20(29)-en-3-one, along with others mentioned in Table 2. The crude methanolic extract of the flower from *E. angustifolius* Blume exhibited no antibacterial or antifungal activity against the investigated bacteria and fungi. This is the first study on the flower extract performed in Nepal. Even though mild biological activities were observed with the extract, it opens a door to further investigation for the isolation and characterization of the active metabolites from the flower, thereby elucidating the mechanisms underlying its antioxidant and antidiabetic properties.

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AUTHOR CONTRIBUTIONS

Conceptualization: SJ; Investigation: RDP; Methodology: SJ; Data curation: SJ; Data analysis: SJ; Writing - original draft: RDP; Writing - review and editing: SJ.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL STATEMENT

This is our original work and has not been previously published or submitted for publication elsewhere.

DATA AVAILABILITY STATEMENT

The supporting data for these findings are available from the corresponding author upon reasonable request.

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