PLANT SCIENCE TODAY
Vol 12(4): 1-9
https://doi.org/10.14719/pst.6611

eISSN 2348-1900

RESEARCH ARTICLE

Bioactivity surveys of some medicinal plants in Trat Agroforestry Research and Training Station, Trat Province

Wasawat Kiatarkom[1], Srunya Vajrodaya[1] & Sutsawat Duangsrisai[1*]

[1]Department of Botany, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand

*Correspondence email - fscissw@ku.ac.th

Received: 09 December 2024; Accepted: 16 May 2025; Available online: Version 1.0: 11 September 2025; Version 2.0: 06 October 2025

Cite this article: Wasawat K, Srunya V, Sutsawat D. ABioactivity surveys of some medicinal plants in Trat Agroforestry Research and Training Station, Trat Province. Plant Science Today. 2025; 12(4): 1-9. https://doi.org/10.14719/pst.6611

Abstract

This study investigates the bioactivity properties of 16 ethanolic extracts from medicinal plants, representing 8 families, collected from the Trat Agroforestry Research and Training Station in Trat province. The research specifically examines three bioactivity properties: antioxidant activity, anti-diabetic activity and anti-inflammatory activity. Antioxidant activity was evaluated using DPPH, FRAP, ABTS and nitric oxide scavenging assays. The anti-diabetic activity was determined by inhibiting alpha-glucosidase and alpha-amylase, while the anti-inflammatory activity was assessed by inhibiting BSA denaturation. The findings indicate that *Volkameria inermis* exhibited the highest antioxidant potential, as measured by DPPH (IC_{50} = 0.011 ± 0.005 mg/mL), ABTS (IC_{50} = 57.173 ± 6.901 mg/mL), FRAP (IC_{50} = 0.070 ± 0.020 mg/mL) and nitric oxide scavenging (IC_{50} = 0.127 ± 0.071 mg/mL) and was characterized by a significant phenolic compound content (121.92 ± 0.66 mg GAE/g). Moreover, extracts from the Garcinia family, specifically *Garcinia cowa* and *Garcinia hanburyi*, demonstrated notable anti-diabetic properties, inhibiting alpha-glucosidase (IC_{50} = 1.383 ± 0.131 mg/mL) and alpha-amylase (IC_{50} = 0.233 ± 0.012 mg/mL), respectively. Notably, *Gnetum gnemon* was the only plant to exhibit substantial anti-inflammatory activity (IC_{50} = 0.034 ± 0.011 mg/mL) through its capacity to protect bovine serum albumin (BSA) from degradation. Consequently, further research is warranted to identify the key substances and mechanisms of action that contribute to the development of effective therapeutic agents and medicinal applications.

Keywords: anti-diabetes; anti-inflammatory; antioxidant; bioassay; phytochemistry; plant extracts

Introduction

The Trat Agroforestry Research and Training Station, located at 37/1 Village Number 8, Tha Kum Subdistrict, Mueang District, Trat Province 23000, represents a significant repository of diverse medicinal plants whose bioactivities remain largely unexplored. This study investigates the bioactivities of selected plants from this unique ecological resource, aiming to fill a critical gap in our understanding of Thai medicinal plants. It is well established that medicinal plants generally possess antioxidant properties, a characteristic commonly found across many plant families. These antioxidant properties play a crucial role in humans by maintaining the balance of free radicals, which helps prevent disorders or diseases such as cancer, diabetes and inflammation (1-4). Moreover, when unbalanced, free radicals can damage cellular function through oxidative stress mechanisms (5). Hence, consuming antioxidants derived from plant extracts is a significant preventive care step.

Thailand's biodiversity presents a rich source of medicinal plants with therapeutic potential, particularly within the Trat Agroforestry Research and Training Station, which contains numerous unstudied medicinal plants. Medicinal plants in Trat Province have the potential to be a source of bioactive compounds with medical applications. Traditional knowledge suggests that medicinal plants may offer advantages over synthetic substances, potentially reducing toxicity while providing therapeutic benefits. Consequently, it is highly advantageous that Thailand possesses this resource for the examination of medicinal plants to expand scientific knowledge.

This study analysed 16 medicinal plants from 8 families collected in the Trat Agroforestry Research and Training Station, Trat Province, Thailand. Each plant family examined represents renowned Thai medicinal plants. Examples include the Acanthaceae family, represented by plants such as *Andrographis paniculata* and *Acanthus montanus*, which are known for medicinal properties including cold relief, immune support and anti-cancer, anti-diabetic and anti-sickness effects (6). The Amaranthaceae family (e.g., *Amaranthus spinosus)* is valued for its antioxidant and anti-inflammatory effects, alongside other medicinal and culinary applications (7, 8). The Rutaceae family includes aromatic plants used in traditional remedies for toothaches and asthma (9). However, these represent only a few examples of well-known Thai medicinal plants. Many more Thai medicinal plants remain unresearched. Additional research into Thai medicinal plants is vital for promoting local alternatives to synthetic medications.

Materials and Methods

Plants sample

All medicinal plant samples in this study (Table 1) were collected from Trat Agroforestry Research and Training Station (12.392010821848181, 102.66834337205964) during 7-8 November 2020 by collecting the whole plant for shrub and collected only leaves for the tree. Each sample was labeled and contained in the zip lock bag. All plant materials were identified and authenticated by Assoc. Prof. Srunya Vajrodaya (Ph.D), Department of Botany, Faculty of Science, Kasetsart University.

Sample drying and sample grinding

After sample collection, all materials were dried at room temperature for 7 days, then each plant sample was ground into a powder with a blender and weighed the powder after finishing grinding.

Sample extraction

The sample powder was macerated in ethanol with a ratio weight by volume 1:10 for 7 days. Then, the sample vial was weighed and the weight was recorded before containing the crude extract. After maceration, the sample solution was evaporated by an evaporator to get rid of all solvent, to get the crude extraction for each sample and transferred into a vial. The vial was weighed again after containing the crude extract and the net weight of each crude sample extract was calculated. All crude extract was kept in the dark at 4 °C for further bioactivity assay.

Chemical reagents

Chemical reagents for this study include 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ethanol, Methanol, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox), Iron(III) chloride, Sodium acetate trihydrate, 37 % HCl, glacial acetic acid, 3,4,5-trihydroxy benzoic acid (gallic acid), quercetin, Folin–Ciocalteu reagent, sodium carbonate, aluminum chloride, Potassium phosphate buffer, alpha-Glucosidase, p-nitrophenyl- α-D-glucopyranoside (PNPG), Sodium carbonate ($Na2CO_3$), Starch solution, Sodium phosphate buffer, alpha-Amylase, Dinitrosalicilic acid (DNS), tricine, calcium chloride, sodium chloride, bovine serum albumin (BSA).

Determination of antioxidant activity

DPPH assay

This study used standard methods from previous reports with some adaptations (10, 11). The fundamental principle underlying this methodology was evaluating antioxidants by measuring their ability to convert a purple 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution through electron donation (12). The yellow solution absorbed light at 520 nm. To prepare samples, 2 mg of each crude extract was dissolved in 1 mL methanol, yielding a concentration of 2 mg/mL, then diluted to 1, 0.5, 0.25 and 0.125 mg/mL. A 0.1 mM DPPH solution was prepared by dissolving 0.7886 mg of DPPH in 20 mL methanol (in the dark). After preparation, 100 µL of each sample concentration and DPPH solution was transferred into a 96-well microplate, using triplicate for each concentration. A sample blank (sample in methanol) and a control blank (DPPH in methanol) were included. The samples were incubated in the dark for 30 minutes, then the absorbance was measured at 520 nm with a microplate reader. Percent inhibition was calculated from the absorbance and the IC_{50} value was determined from the correlation between percent inhibition and sample concentration. The calculation of percent inhibition employed a consistent equation across all experiments, which was described in further detail. In this study, the percentage inhibition was determined utilizing the following equation.

Percent inhibition =

$$(Abs_{control}-(Abs_{sample}-Abs_{sample\ blank})/Abs_{control})*100$$

Ferric Reducing Antioxidant Power (FRAP)

The core reagent for this experiment was the FRAP solution. This methodology was conducted according to procedures previously reported (13). The FRAP solution was freshly prepared by mixing the three solutions including 300 mM acetate buffer, 10 mM TPTZ and 20 mM $FeCl_3$. The 15 µL of methanolic plant samples extracted in different concentrations and 285 µL of FRAP solution were transferred into a microplate using a micropipette and kept in the dark for 30 minutes, then the microplate was placed into a microplate reader and measured at wavelength 593 nm. The standard Trolox was also carried out in the same way. Each experiment

Table 1. List of medicinal plants for the study

Common name	Scientific name	Family	Part used
Phuk Kraad Talay (ผักคราดทะเล)	*Wedelia biflora*	Asteraceae	Whole plants
Phuk Kraed (ผักแครด)	*Synedrella nodiflora*	Asteraceae	Whole plants
Phuk Krad Hua Waan (ผักคราดหัวแหวน)	*Acmella oleracea*	Asteraceae	Whole plants
Khee Kai Yaan (ขี้ไก่ย่าน)	*Mikania cordata*	Asteraceae	Whole plants
Sarb Rang Sarb Ga (White florets) (สาบแร้งสาบกาดอกสีขาว)	*Ageratum conyzoides*	Asteraceae	Whole plants
Sarb Rang Sarb Ga (Purple florets) (สาบแร้งสาบกาดอกสีม่วง)	*Ageratum conyzoides*	Asteraceae	Whole plants
Sarb Muang (สาบม่วง)	*Praxelis clematidea*	Asteraceae	Whole plants
Rong Thong (รงทอง)	*Garcinia hanburyi*	Clusiaceae	Leaves
Cha Muang (ชะมวง)	*Garcinia cowa*	Clusiaceae	Leaves
San Soak (สันโสก)	*Clausena excavata*	Rutaceae	Leaves
Keuy Taay (เขยตาย)	*Glycosmis pentaphylla*	Rutaceae	Leaves
Ya Yaa (หญ้าหยา)	*Asystasia gegantica*	Acanthaceae	Whole plants
Yaa Pun Nguu (หญ้าพันงู)	*Achyranthes aspera*	Amaranthaceae	Whole plants
Phuk Liang (ผักเหลียง)	*Gnetum gnemon*	Gnetaceae	Leaves
Sam Ma Nga (ส้มะงา)	*Volkameria inerme*	Lamiaceae	Leaves
Yha Nhon Taay (หญ้าหนอนตาย)	*Pouzolzia pentandra*	Utricaceae	Whole plants

was carried out in triplicate and the mean absorbance was recorded. The content within the analysed sample was expressed as mg of trolox equivalent (TE) per gram of extract.

ABTS assay

For the ABTS assay, the methodology followed the protocol reported, with some modifications (14). For this study, the 7 mM stock solution of ABTS.+ and 2.45 mM potassium persulfate was prepared in a 1:1 ratio and allowed to react in the dark for 12-16 hr. Before use, the stock solution was diluted with methanol to absorb approximately 0.7 ± 0.05 at 734 nm. A total of 20 µL of the sample at varying concentrations was combined with 180 µL of the ABTS.+ solution in a 96-well microplate, with water used for sample blanks and allowed to react for 45 sec before incubating at room temperature for 15 min. Absorbance was measured at 734 nm using a microplate reader. For the control, 180 µL of ABTS.+ solution was mixed with 20 µL of methanol. Each experiment was conducted in triplicate and the absorbance values were expressed as percent inhibition and converted to IC_{50} values.

Nitric Oxide (NO) scavenging assay

The ability to scavenge nitric oxide was assessed by the previous report (15). This study focused on the ability of plant sample extracted could scavenge colorimetric NO metabolite in the pink form generated from sulfanilamide reacting with NO_2 released from sodium nitroprusside dihydrate (SNP) and N-(1-naphthyl) ethylenediamine. The 0.1 M stock of phosphate buffer was prepared and pH was adjusted to 7.4 and stored at 4 °C. 20 mM of sodium nitroprusside dihydrate played as a NO donor and was prepared by dissolving in phosphate buffer. The stock of Griess reagent was also prepared by dissolving N-(1-naphthyl) ethylenediamine dihydrochloride and sulfanilamide with 2 % phosphoric acid (H_3PO_4). In the sample step, 100 µL of plant samples extracted in each different concentration and SNP were transferred into a 96-well microplate and kept under light for 60 min, then Griess reagent (H_3PO_4 for sample blank) was added into each well-containing sample extracted and SNP. The samples were kept in the dark for 10 min and absorbance was measured at 560 nm. The experiment was performed in triplicate and the results were reported as IC_{50} value which was calculated from percent inhibition.

Total Phenolic Contents (TPC) and Total Flavonoid Contents (TFC) assay

TPC and TFC assays were essential for evaluating total antioxidant capacity by measuring phenolic and flavonoid contents in plant extracts against standard reagents. Gallic acid and quercetin were used as standards for TPC and TFC, respectively (16). The TPC assay involved diluting 0.2 M Folin-Ciocalteu's reagent and dissolving sodium carbonate in water. A plant extract was prepared at 1 mg/mL and varying concentrations of gallic acid (200, 100, 50, 25, 12.5 µg/mL) were dissolved in methanol for the standard. Each concentration was added to a 96-well microplate with the reagent and sodium carbonate, with absorbance measured at 765 nm, targeting a standard curve absorbance of 0-1. The same procedure was applied to the plant extract, with absorbance expected to range from 0.2-0.8, while the blank included gallic acid and the sample extract reacting with water. In the TFC assay, a 2 % w/v aluminium chloride ($AlCl_3$) stock solution was prepared in methanol, with the plant extract again at 1 mg/mL. Varying concentrations of quercetin (50, 25, 12.5, 6.25, 3.125 µg/mL) were dissolved in methanol for standard preparation. In a 96-well microplate, 100 µL of the AlCl3 solution was added followed by the plant extract, incubated for 15 min at room temperature and absorbance was measured at 415 nm. Quercetin replaced the plant extract in the standard method, while both were mixed with methanol in the blank. Absorbance for the sample extract and quercetin should have been approximately 0.2-0.8 and 0-1, respectively. The assay was performed in triplicate, with results expressed as gallic acid equivalents for TPC and quercetin equivalents for TFC.

Determination of anti-diabetic activities

Alpha-glucosidase inhibition activities assay

Alpha-glucosidase was an enzyme that catalysed the hydrolysis of disaccharides and oligosaccharides into glucose (17). In this study, p-nitrophenyl-α-D-glucopyranoside (pNPG) served as the substrate and was hydrolysed to p-nitrophenol, which was measured at a wavelength of 405 nm. A 100 mM potassium phosphate buffer (pH 6.8) was prepared by mixing 1 M monopotassium phosphate and 1 M dipotassium phosphate in equal proportions. A 2 mM pNPG solution was made using the potassium phosphate buffer, while a fresh 1 U/mL alpha-glucosidase solution was also prepared in the same buffer. Additionally, a 0.1 mM sodium carbonate solution was prepared in water. For the assay, 20 µL of the plant extract (or buffer for controls) was added to a 96-well microplate, followed by 50 µL of potassium phosphate buffer to each well. Next, 10 µL of the alpha-glucosidase solution (or buffer for blanks) was added and the mixture was incubated at 37 °C for 15 min. Subsequently, 20 µL of pNPG was added to each well, followed by another incubation at 37 °C for 15 min. Finally, 50 µL of 0.1 M sodium carbonate was added and the absorbance was measured at 405 nm using a microplate reader. The experiment was conducted in triplicate and the percent inhibition was calculated to derive the IC_{50} value.

Alpha-amylase inhibition activities assay

Alpha-amylase and alpha-glucosidase enzymes had similar functions but distinct properties. Alpha-amylase hydrolysed long-chain carbohydrates, such as starch, into smaller units like oligosaccharides, whereas alpha-glucosidase converted disaccharides into glucose. Alpha-amylase was in the salivary glands and pancreas, while alpha-glucosidase was found in the epithelial cells of the small intestine (18). For the experiment, a sodium phosphate buffer (20 mM, pH 6.9) was prepared by dissolving 1.693 g of Na_3PO_4 and 175.2 mg of NaCl in water, adjusting the pH to 6.9 and bringing the volume to 500 mL. A 1 % starch solution was made by dissolving 0.5 g of starch in 50 mL of warm sodium phosphate buffer. Alpha-amylase was prepared at 0.5 mg/mL concentration in the buffer. A Dinitrosalicilic acid (DNS) solution was created by mixing 1 g of DNS, 20 mL of 2M NaOH (prepared by dissolving 8 g NaOH in 100 ml water) and 30 g of potassium sodium tartrate. In the experimental procedure, 200 µL of the plant extract (for the sample and sample blank) or sodium phosphate buffer (for the control and control blank) was added to a microcentrifuge tube,

followed by 200 µL of starch solution. The mixture was incubated for 10 min at 25 °C. Subsequently, 200 µL of alpha -amylase solution (for sample and control steps) or sodium phosphate buffer (for blanks) was added and the mixture was incubated again for 10 minutes at 25 °C. Finally, 400 µL of DNS solution was added to each tube and then incubated at 100 °C for 5 min. After cooling, 50 µL of solution from each tube was transferred to a 96-well microplate, followed by the addition of 200 µL of water and absorbance was measured at 540 nm using a microplate reader. The experiment was performed in triplicate to calculate percent inhibition and determine IC_{50} values.

Determination of anti-inflammatory activity

Protein denaturation is one cause of inflammation (18). The inhibition of protein denaturation serves as a method to evaluate anti-inflammatory activities (19). Therefore, this study needed to learn about the potential of samples extracted to inhibit protein breakdown and further develop into anti-inflammatory drugs. The experimental method was adapted from (20) and (21). First, a 10X tris buffer saline (TBS) stock solution was prepared by dissolving 12 g of tris base and 44 g of NaCl. The pH was adjusted to 7.6, then the final volume was adjusted to 500 mL with distilled water. Next, 50 mL was transferred from the 10X TBS stock solution and diluted with 450 ml distilled water to acquire a 1X TBS working solution. Fifty ml of 0.2% w/v bovine serum albumin (BSA) was prepared by dissolving 0.1 g BSA in 50 mL of 1X TBS solution. One hundred fifty µL of each different concentration of samples extracted (1X TBS for the control step) was transferred to a microcentrifuge tube, followed by 150 µl of 0.2 % w/v BSA. The mixture was heated at 72 °C for 10 min and then allowed to cool for 20 min. Finally, the solution from each single tube was transferred to a 96-well microplate and absorbance was measured at 660 using a microplate reader. Sample blank and control blank were also carried out and the experiment was performed in triplicate and calculated for percent inhibition and finalized the results as IC_{50} value.

Statistical analysis

All tests were performed for 3 triplicates per experiment. The results are expressed as mean value ± SD (n=3). The difference among the treatments were determined, using the analysis and LSD test for the post-hoc comparison. The means deferring at the $p \leq 0.05$ were considered significant differences. The analysis was performed using SPSS.

Result and Discussion

Determination of antioxidant activities

According to the results derived from the ethanolic extraction of 16 medicinal plants, as shown in Table 2. The findings are represented by the IC_{50}, FRAP, TPC and TFC values. Volkameria inerme demonstrated significant antioxidant activity through low IC_{50} values and high FRAP results. In terms of total phenolic content, Volkameria inerme had a high concentration of phenolic compounds compared to others but was lower than Pouzolzia pentandra, which had the highest. Various antioxidants exhibit precursor free radicals in DPPH, FRAP, ABTS and nitric oxide assays. The study indicates the presence of multiple compounds in the ethanolic extract of Volkameria inerme that stabilize free radicals, positioning it second among the 16 samples analysed. This suggests the potential for various phenolic compounds to neutralize different free radicals in reactions. Despite Pouzolzia pentandra being expected to have the highest phenolic content, its antioxidant properties are less pronounced than those of Volkameria inerme. While phenolic compounds are common in medicinal plants (22), the phenolic assessment from Pouzolzia pentandra may show limited interaction with the antioxidant methodologies applied in this research.

The findings of this investigation regarding the antioxidant properties of Volkameria inerme align, highlighting its rich composition of crucial antioxidants, particularly acteoside (23). Additionally, leaf extracts demonstrate significant antioxidant activities, with elevated DPPH and ABTS radical scavenging capabilities (24, 25). Nevertheless, the antioxidant potential of Volkameria inerme utilizing FRAP assay and nitric oxide scavenging mechanisms remains underexplored, presenting an opportunity for further research. This suggests that the findings from the FRAP analysis and nitric oxide scavenging studies could open new inquiries into the constituents of this medicinal plant. Furthermore, the TFC analysis revealed that the methanolic extract of Volkameria inerme contained fewer flavonoid compounds compared to extracts from other plants. Specifically, the quercetin-related concentration was recorded at 10.77 ± 0.61 mg QE/g, while Achyranthes aspera exhibited the highest flavonoid concentration at 66.71 ± 0.13 mg QE/g. Importantly, the TFC results for Volkameria inerme were consistent with previous studies. A flavonoid content of 16.65 ± 1.59 mg QE/g in the leaf extract of Volkameria inerme, alongside a phenolic content of 58.55 ± 3.19 mg GAE/g was reported (26). The current study demonstrated that Volkameria inerme possessed a greater number of phenolic compounds, while flavonoid levels remained comparable. However, there is a paucity of research regarding the phenolic and flavonoid compounds in Volkameria inerme and specific antioxidant compounds have yet to be identified. Further investigations and reviews of pertinent literature will likely be warranted shortly.

Determination of anti-diabetes activities

In this investigation, the compound's anti-diabetic efficacy was evaluated by its capability to inhibit alpha-amylase and alpha-glucosidase activity, as shown in Table 3. Only *Garcinia cowa* and *Garcinia hanburyui* exhibited significant IC50 values for alpha-amylase inhibition. A different extract showed inhibition levels independent of its concentration. Multiple *Garcinia* species demonstrate notable anti-diabetic effects. *Garcinia atroviridis* can reduce blood sugar levels and inhibit both alpha-amylase and alpha-glucosidase activities (27). Extracts from *Garcinia xanthochymus*, *Garcinia kola* and *Garcinia linii* are recognized for their blood sugar regulation and amylase inhibition. Anti-diabetic compounds such as flavonoids and xanthones not only exhibit anti-diabetic effects but also enhance lipid metabolism and regulate blood sugar levels. These compounds also improve insulin sensitivity and alleviate diabetic complications (28, 29).

Table 2. Determination of antioxidants activity from ethanolic extract from 16 studied medicinal plants

Scientific name	Family	Antioxidant activity					
		DPPH (IC_{50}, mg/mL)	FRAP (mgTE/g)	ABTS (IC_{50}, mg/mL)	NO scavenging (IC_{50}, mg/mL)	TPC (mg GAE/g)	TFC (mg QE/g)
Wedelia biflora	Asteraceae	0.197±0.467^{ab}	3.990±1.300^{b}	0.437±0.119^{ab}	0.673±0.318^{ab}	12.337±1.994^{j}	27.483±1.470^{c}
Synedrella nodiflora	Asteraceae	0.127±0.003^{a}	5.917±3.723^{b}	0.217±0.437^{a}	0.380±0.142^{ab}	27.980±0.431^{gh}	19.270±0.455^{efg}
Acmella oleracea	Asteraceae	0.170±0.045^{a}	11.473±0.639^{b}	0.437±0.064^{ab}	0.277±0.89^{ab}	76.000±0.509^{d}	29.537±0.439^{c}
Mikania cordata	Asteraceae	0.233±0.186^{ab}	15.047±2.285^{b}	0.233±0.054^{ab}	0.183±0.059^{a}	67.917±1.273^{d}	37.053±0.667^{b}
Ageratum conyzoides	Asteraceae	0.403±0.028^{abc}	14.860±3.521^{b}	0.603±0.071^{b}	0.403±0.027^{ab}	25.407±0.302^{h}	25.353±0.255^{cd}
Ageratum conyzoides	Asteraceae	0.920±0.204^{c}	14.123±2.429^{b}	0.360±0.085^{ab}	0.920±0.204^{ab}	35.070±0.557^{fg}	9.050±0.185^{h}
Praxelis clematidea	Asteraceae	0.127±0.018^{a}	11.100±0.985^{b}	0.167±0.017^{a}	0.103±0.032^{a}	48.217±4.063^{e}	16.997±0.521^{fg}
Garcinia hanburyi	Clusiaceae	0.150±0.231^{a}	14.317±2.655^{b}	0.187±0.138^{a}	0.150±0.023^{a}	110.310±2.156^{c}	20.993±0.214^{def}
Garcinia cowa	Clusiaceae	0.310±0.093^{ab}	11.863±1.674^{b}	0.283±0.003^{ab}	0.310±0.093^{ab}	36.917±1.770^{f}	22.353±0.141^{de}
Clausena excavata	Rutaceae	0.707±0.314^{bc}	6.087±0.630^{b}	0.223±0.078^{ab}	0.707±0.314^{ab}	43.173±0.290^{ef}	2.947±0.056^{i}
Glycosmis pentaphylla	Rutaceae	0.127±0.009^{a}	13.733±0.861^{b}	0.200±0.082^{a}	0.170±0.035^{a}	36.663±1.029^{f}	21.237±1.041^{def}
Asystasia gegantica	Acanthaceae	0.150±0.025^{a}	11.630±0.982^{b}	0.217±0.067^{a}	0.140±0.032^{a}	26.793±1.087^{h}	16.207±1.126^{g}
Achyranthes aspera	Amaranthaceae	0.470±0.012^{abc}	8.317±2.678^{b}	0.2033±0.098^{a}	0.470±0.011^{ab}	15.907±0.037^{ij}	66.713±0.003^{a}
Gnetum gnemon	Gnetaceae	0.183±0.231^{a}	5.300±0.759^{b}	0.267±0.059^{ab}	0.273±0.075^{ab}	20.540±0.992^{hi}	21.437±0.361^{def}
Volkameria inerme	Lamiaceae	0.011±0.005^{a}	57.173±6.901^{a}	0.070±0.020^{a}	0.127±0.071^{a}	121.922±0.380^{b}	10.770±0.348^{h}
Pouzolzia pentandra	Utricaceae	0.223±0.145^{ab}	48.760±7.871^{a}	0.137±0.024^{a}	0.217±0.020^{ab}	193.232±2.052^{a}	22.150±2.360^{de}

Garcinia cowa is a medicinal plant known for its anti-diabetic efficacy, supported by previous studies. Study indicates that ethyl acetate extracts from *Garcinia cowa* stem bark have an IC50 of 12.54 ± 0.49 µg/mL for alpha-amylase inhibition (30). The ethanolic leaf extract exhibited an IC50 of 28.22 ± 0.39 µg/mL, which is considered superior in this study. Other studies highlight the role of flavonoids and triterpenoids in regulating blood glucose and lipid metabolism (31). Furthermore, the antioxidant properties of *Garcinia cowa* significantly enhance its anti-diabetic potential. A report revealed that methanolic leaf extracts exhibit strong antioxidant activity, with an EC50 of 1597.5 ± 99.3 µg/mg and phenolic content of 36.91 ± 3.07 mg/g, surpassing previously reported values in this study (32).

Moreover, a new compound from Garcinia cowa has been discovered. *Garcinia cowone* K (Fig. 1), a decahydro-1H-xanthene derivative, exhibits antidiabetic properties. This compound features several functional groups, such as carbonyl and benzoyl, contributing to its bioactivity. The carbonyl groups in *Garcinia cowone* K play a key role in inhibiting the alpha-glucosidase enzyme, which is vital for glucose metabolism regulation. These functional groups likely interact with the enzyme's active site, leading to inhibitory effects and glucose modulation (33). Consequently, *Garcinia cowa* exhibits sufficient anti-diabetic properties for potential drug development.

In terms of anti-diabetic effects via alpha-glucosidase inhibition, *Garcinia hanburyi* extract shows superior enzyme inhibitory properties with an IC_{50} of at least 0.03 ± 0.02 mg/mL, indicating its anti-diabetic similarities with other G*arcinia* species. Nonetheless, research on the anti-diabetic effects of *Garcinia hanburyi* leaf extract remains limited, primarily focusing on gamboge, a resin from the plant. Gamboge contains key compounds like gambogoic acid, moreollic acid, gambogic acid and 10-methoxygogenic acid (Fig. 2). These compounds significantly inhibit alpha glucosidase, with methoxy group of ring A and pentenyl pyran moiety of ring D in gambogoic acid exhibiting the highest inhibitory activity (34).

Previous investigations have examined alpha-glucosidase inhibition. A study explored the bioactivities of *Garcinia mckeaniana* leaf extracts, revealing the presence of various xanthone derivatives, including norathyriol, which exhibits significant alpha-glucosidase inhibitory effects with an IC_{50} of 0.07 µM (35). Additionally, *Volkameria inerme* shows comparable alpha-glucosidase inhibition to *Garcinia hanburyi,* with similar IC_{50} values (0.12 ± 0.03 mg/mL), possibly due to its antioxidant properties and high phenolic compound levels. However, research on the anti-diabetic properties of *Volkameria inerme* remains scarce. Further investigation into the alpha-glucosidase inhibitory properties of *Garcinia hanburyi* and *Volkameria inerme* leaf extracts is warranted, particularly regarding solvent effects, isolation of active compounds, structural analysis and potential applications in diabetes treatment development.

Determination of anti-inflammatory activities

In the anti-inflammatory assessment, only the *Gnetum gnemon* extract yields an IC_{50} inhibition percentage value. Other extracts cannot derive this value due to factors observed in prior experiments. Certain extracts display inhibition percentages that are not concentration-dependent, with higher concentrations sometimes resulting in negative inhibitions. Additionally, negative percentage inhibitions are also observed like the study, possibly due to experimental errors or some condition (21). This suggests the tested extracts may not effectively inhibit denaturation and could, in some instances, promote it. Such results may stem from inadequate maturation of the extract prior to experimentation, leading to component degradation or the presence of inhibitors resistant to denaturation. Some extracts do exhibit anti-denature effects on BSA at low concentrations, consistent with findings from (36) regarding *Boehmeria jamaicensis* leaf extract. BSA denaturation is substantially prevented at concentrations below 0.012 ug/mL, while higher concentrations inversely affect efficacy. Additionally, extracts with non-diluted suppression rates resemble results which examined 99 Jamaican plant extracts, noting variable inhibition tendencies (20). This indicates that the ability of these extracts to prevent protein denaturation is not solely dosage-dependent,

Table 3. Determination of anti-diabetes and anti-inflammatory activity from ethanolic extract from 16 studied medicinal plants

Scientific name	Family	Anti-diabetes activity		Anti-inflammatory activity (IC_{50}, mg/mL)
		Alpha-amylase inhibition (IC_{50}, mg/mL)	Alpha-glucosidase inhibition (IC_{50}, mg/mL)	
Wedelia biflora	Asteraceae	N/A	1.030±1.000[a]	N/A
Synedrella nodiflora	Asteraceae	N/A	0.103±0.083[a]	N/A
Acmella oleracea	Asteraceae	N/A	0.360±0.260[a]	N/A
Mikania cordata	Asteraceae	N/A	0.100±0.023[a]	N/A
Ageratum conyzoides	Asteraceae	N/A	1.670±1.600[a]	N/A
Ageratum conyzoides	Asteraceae	N/A	1.730±1.640[a]	N/A
Praxelis clematidea	Asteraceae	N/A	0.637±0.607[a]	N/A
Garcinia hanburyi	Clusiaceae	0.892±0.179	0.233±0.012[a]	N/A
Garcinia cowa	Clusiaceae	1.383±0.131	0.173±0.083[a]	N/A
Clausena excavata	Rutaceae	N/A	0.507±0.467[a]	N/A
Glycosmis pentaphylla	Rutaceae	N/A	0.243±0.167[a]	N/A
Asystasia gegantica	Acanthaceae	N/A	0.227±0.037[a]	N/A
Achyranthes aspera	Amaranthaceae	N/A	0.350±0.163[a]	N/A
Gnetum gnemon	Gnetaceae	N/A	0.543±0.504[a]	0.034±0.011
Volkameria inerme	Lamiaceae	N/A	0.117±0.176[a]	N/A
Pouzolzia pentandra	Utricaceae	N/A	0.100±0.020[a]	N/A

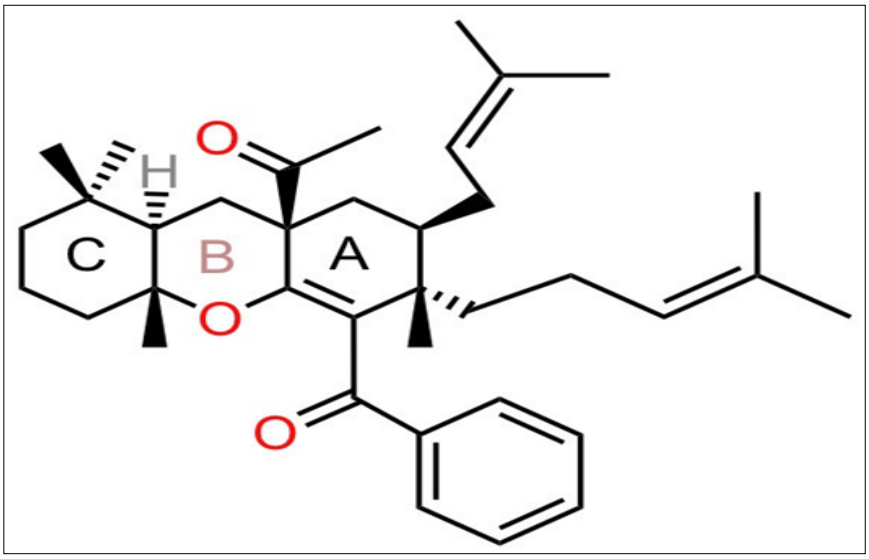

Fig. 1. Structure of *Garcinia cowone* K.

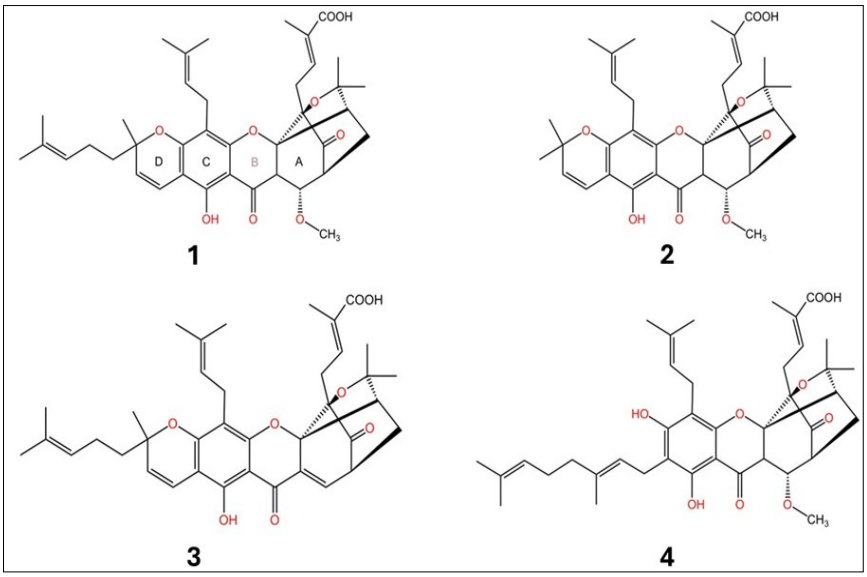

Fig. 2. The structure of compounds (1-4) which were isolated from gamboge identified as (1) gambogoic acid, (2) moreollic acid, (3) gambogic acid and (4) 10-methoxygambogenic acid.

maintaining consistent inhibition across assessed dosages. Independent dosage inhibition could enhance pharmaceutical research by ensuring uniform effectiveness of compounds within specific dosage ranges, thereby improving therapeutic reliability.

The anti-inflammatory effects of *Gnetum gnemon* on BSA denaturation remain unexplored. Prior studies have indicated that its leaf extracts possess anti-inflammatory properties via lipoxygenase and cyclooxygenase inhibition (37). The bioactive constituents of *Gnetum gnemon*, such as saponins, tannins, flavonoids and phenolic compounds, demonstrate notable anti-inflammatory effects (38). Another study revealed that an ethanol extract of *Gnetum gnemon* leaves contains stilbenoid compounds that significantly reduce nitric oxide synthesis, a major inflammatory mediator (39, 40). There is potential for *Gnetum gnemon* to provide considerable anti-inflammatory benefits, though further research is essential to confirm this in future investigations. Future studies are also required to explore the compounds responsible for BSA denaturation inhibition in *Gnetum gnemon*. (41) suggested that flavonoids may play a significant role in preventing BSA denaturation. However, the mechanisms by which flavonoids influence heat-induced BSA denaturation remain unexplained, while it was proposed by a study that these compounds may interact with various binding sites on BSA to maintain its structural integrity during denaturation (36).

Conclusion

The evaluation of the antioxidant, anti-diabetic and anti-inflammatory activities of 16 medicinal plant extracts using ethanol as a solvent. This study revealed that *Volkameria inermis* is a plant that showcases a wide range of biological properties. Through many experiments, it has been established that this plant possesses antioxidant properties, as evidenced by outcomes from DPPH, ABTS, FRAP and nitric oxide scavenging methodologies, as well as its abundance in phenolic and flavonoid compounds. Moreover, its promising antioxidation properties have the potential and contribute to many other positive biological effects, encompassing anti-diabetic and anti-inflammatory effects. Additionally, the *Garcinia* genus, including *Garcinia cowa* and *Garcinia hanburyi* have been identified as a promising sample of investigation regarding their anti-diabetic properties and showing potential to develop as an antidiabetic drug., while *Gnetum gnemon* in

this study exhibit the potential as an anti-inflammatory agent. However, the analysis of crucial phytochemical compounds in various plant types and the study of the mechanisms of action of those compounds in various acts still require further research in the future. Especially the anti-inflammatory properties of *Gnetum gnemon*, which in this report may be the first to report results using BSA denaturation methods, which is expected this report might be good information for further studies.

Acknowledgements

Authors thank to Suphitsara Pansiri for evaporation process, bioactivities process and scientific advice. Authors thank Pajaree Sathupun for assistance with statistical analysis. Authors thank Pakawat Jarupund for assisting in verifying the template to ensure compliance with PST guidelines. Authors thank to the Scientific Equipment Center, Faculty of Science, Kasetsart University for support on Microplate Reader studies. Authors thank to Development and Promotion of Science and Technology Talents Project (DPST) Scholarship, Royal Government of Thailand for funding support. Authors also thank to Puntaree Meemook, the members of the Phytochemistry Research Unit and other members of Department of Botany, Faculty of Science, Kasetsart University for their friendship, support and encouragement.

Authors' contributions

WK performed the experiments, data analysis and drafted the manuscript. SV contributed to provide important suggestions related to research. SD performed the research concept and approved of the article. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

References

1. Moeiklang N, Ruangviriyachai. Determination of phenolic compounds and antioxidant potential in fruit beverages. Asia Pacific J Sci Tech 2015;14(4)69-79.
2. Ali A, Kumar M, Srivastava N, Khan MM, Khan MA. Free radicals and *Diabetes mellitus.* Int J Pharm Sci Med. 2023;8(3):1-19. https://doi.org/10.47760/ijpsm.2023.v08i03.001
3. Closa D, Folch-Puy E. Oxygen free radicals and the systemic inflammatory response. IUBMB Life J. 2004;56(4):91-185. https://doi.org/10.1080/15216540410001701642
4. Winrow PGW, Morris CJ, Blake DR. Free radicals in inflammation : second messengers and mediators of tissue destruction. Br Med Bull. https://doi.org/10.1093/oxfordjournals.bmb.a072627
5. Antolovich M, Prenzler PD, Patsalides E, McDonald S, Robards K. Methods for testing antioxidant activity Anal. 2022;127(1):98-183. https://doi.org/10.1039/B009171P
6. Manjula M, Sankar DS. Review on biological activities in medicinal plants of Acanthaceae family. Int J Res Pharma Sci 2021;12(1)33-315. https://doi.org/10.26452/ijrps.v12i1.4296
7. Asha S, Rekha R, Sadiq AMM. A*maranthus spinosus*- A review. 2016
8. Eshete MA, Asfaw ZA, Kelbessa E. A review on taxonomic and use diversity of the family Amaranthaceae in Ethiopia. J Med Plants Stud. 2016:4;94185.
9. Zohora FT, Hasan CM, Ahsan M. Chemical constituents, cytotoxic activities and traditional uses of *Micromelum minutum* (Rutaceae): review. Pharma Pharmaco Int J. 2019;7(5):229-3600.
10. Iqbal E, Salim KA, Lim LBL. Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniothalamus velutinus* (Airy Shaw) rom Brunei Darussalam. J King Saud Univ Sci 2015;27(3)32-224. https://doi.org/10.1016/j.jksus.2015.02.003
11. Choudhary PK, Bhatia AK, Goel A. Efficacy of antimicrobial and antioxidant activity of *Solanum xantocarpum* whole plant hot aqueous extracts. J Appl Microbiol. 2016;10(2):1403-08.
12. Burman S, Chandra G. Phytochemical screening and *in-vitro* antibacterial and DPPH free radical scavenging activities of methanol extract of root of *Combretum album* Pers. Plant Sci Today. 2021;8(4):9-820. https://doi.org/10.14719/pst.2021.8.4.1158
13. Suksungworn R, Duangsrisai S. Phytochemical contents and antioxidant activity of medicinal plants from the Rubiaceae family in Thailand. Plant Sci Today. 2021;8(1):24-31. https://doi.org/10.14719/pst.2021.8.1.882
14. Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byrne DH. Comparison of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts. J Food Compos Anal. 2006;19(6-7):75-669. https://doi.org/10.1016/j.jfca.2006.01.003
15. Songserm R, Kaeoboon S, Suksungworn R, Duangsrisai S, Sanevas N. GC-MS profiling, anti-oxidant and anti-diabetic assessments of extracts from microalgae *Scenedesmus falcatus* (K U B_1) and *Chlorella sorokiniana* (K.U.B_2). Plant Sci Today 2002;9 (3);41-632.
16. Shahidi F, Zhong Y. Measurement of antioxidant activity. J Funct Foods 2015;18:81-757. https://doi.org/10.1016/j.jff.2015.01.047
17. Athipornchai A, Homvisasevongsa S, Semsri S. Discovery and development of Thai medicinal plants with potential antidiabetic activity. Faculty of Science, Burapha University; 2019.
18. Almira D, Subaidah WA, Ananto AD, Deccati RF, Muliasari H. *In vitro* concentration optimization of ethanol extract from Makasar fruit seeds (*Brucea javanica* L. Mern) as an anti-inflammatory agent. J Pijar MIPA 2021;16(5)9-595. https://doi.org/10.29303/jpm.v16i5.2655
19. Waleed A, Thippeswamy NB, Saif ASM, Rajeshwara A. Evaluation of *in vitro* anti-inflammatory and antioxidant activities of *Simarouba glauca* leaf extract. Plant Sci Today 2025;12(1):1-9. https://doi.org/10.14719/pst.3447
20. Yvonne A, Bailey-Shaw LADW, Cheryl E, Green, Rodney S, Smith AM. *In-vitro* evaluation of the anti-inflammatory potential of selected Jamaican plant extracts using the bovine serum albumin protein denaturation assay. Int J Pharm Sci Rev Res 2017;47(1)53-145.
21. Mouffouk C, Hambaba L, Haba H, Mouffouk S, Bensouici C, mouffouk S, et al. Acute toxicity and *in vivo* anti-inflammatory effects and *in vitro* antioxidant and anti-arthriti-c potential of *Scabiosa stellata.* Orient Pharm Exp Med. 2018;18(4):48-335. https://doi.org/10.1007/s13596-018-0320-3

22. Sun W, Shahrajabian MH. Therapeutic potential of phenolic compounds in medicinal plants-natural health products for human health. Mol. 2023;28(4). https://doi.org/10.3390/molecules28041845

23. Chan EWC, Wong SK, Chan HT. *Volkameria inermis:* In overview of its chemical constituents and pharmacologic al properties, notably the amelioration of motor tics. J Herbmed Pharmacol. 2023;12(2):86-176. https://doi.org/10.34172/jhp.2023.18

24. Selvaraj G, Kaliamurthi S, Umamaheswari G, Shanmugapriya R. Antioxidant and radical scavenging effect of *Clerodendrum inerme* (L). World J Fish Marine Sci. 2010;2(1);9-66.

25. Thiripura SU, Shanthi P. Molecular characterization and antioxidant activity of *Volkameria inermis* L. Curr Bot. 2022;4652. https://doi.org/10.25081/cb.2022.v13.7628

26. Manjulatha K, Gul MZ, Imam N, Ghazi IA, Setty OH. Phytochemical content and antioxidant potential of *Clerodendrum inerme* and its different parts-A comparative study. J Bio Active Prod Nat 2016:6 (1);65-77. https://doi.org/10.1080/22311866.2016.1162111

27. Anawat Kongchian NK, Boonrak T, Lookyee S, Buasri K, Surongkul N, Tangpong J. Anti-hyperlipidemia and anti-obesity properties of *Garcinia atroviridis* and *Camellia sinensis* extracts in high-fat diet mice. Walailak J Sci Tech 2020:17(10).https://doi.org/10.48048/wjst.2020.10717

28. Chen TH, Tsai MJ, Fu YS, Weng CF. The exploration of natural compounds for anti-diabetes from distinctive species *Garcinia linii* with comprehensive review of the Garcinia family. Biomolecules 2019;9(11). https://doi.org/10.3390/biom9110641

29. Payamalle SKSJ, Bijjaragi SC, Aware C, Jadhav JP, Murthy HN. Anti -diabetic activity of *Garcinia xanthochymus* seeds. Comp Clin Path 2017:26(2);46-437. https://doi.org/10.1007/s00580-016-2396-9

30. Wanna C, Boonman N, Phakpaknam S. Antioxidant and antidiabetic properties of *Garcinia cowa* Roxb. extracts from leaves, fruit rind and stem bark in different solvents. Plant Sci Today. 2023;10(3). https://doi.org/10.14719/pst.2455

31. Pathak KRS, Das A. Unlocking the therapeutic potential of *Garcinia cowa* Rox. In diabetes management. Sci Phy 2023:2(1)38-41. https://doi.org/10.58920/sciphy02010038

32. Rahmaddian T. Antioxidant potential of *Garcinia cowa* Roxb.; A review. Int J Pharm Sci Med 2023:7(9);6-20. https://doi.org/10.47760/ijpsm.2022.v07i09.003

33. Phukhatmuen P, Raksat A, Laphookhieo S, Charoensup R, Duangyod T, Maneerat W. Bioassay-guided isolation and identification of antidiabetic compounds from *Garcinia cowa* leaf extract. Heliyon 2020:6(4);e03625. https://doi.org/10.1016/j.heliyon.2020.e03625

34. Jin YM, Kim JY, Lee SM, Tan XF, Park KH. α-Glucosidase inhibitory caged xanthones from the resin of *Garcinia hanburyi.* J Appl Biol Chem 2019;62(1)6-81. https://doi.org/10.3839/jabc.2019.012

35. Thu TN, Minh QPM, Van PC, Van NT, Van PK, Thanh NT, et al. Cytotoxic and α-glucosidase inhibitory xanthones from *Garcinia mckeaniana* leaves and molecular docking study. Chem Biodivers 2021;18(11):1-9. https://doi.org/10.1002/cbdv.202100396

36. Williams AOC, Latore L, Dennis O, Ringer S, Whittaker JA, Conrad J, et al. The *in vitro* anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (mmunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals, in the early stages of the drug discovery process. West Indian Med J.2008;57(4):31-327.

37. Herlina, Indah S, Dian PW, Nuttahmah AN. Antidiarrheal activity of extract ethanol melinjo leaves (*Gnetum gnemon* (L.) Linn.) In wistar white rats induced by *Escherichia coli* and extract standardization. Int J Appl Pharm 2022;14(3):6-620. https://doi.org/10.22159/ijap.2022.v14s3.12

38. Ching HY, Binti NNA. Determination of selected phytochemicals and nutritional compositions in melinjo (G*netum gnemon* L) fruit using different extraction solvents. J Agri Res 2022;7(2):1-7. https://doi.org/10.23880/oajar-16000290

39. Partha P, Dutta MB, Sonali R, Bardwi N, Kabita G, Dibya R, et al. Antiplasmodial activity of *Gnetum gnemon* leaves and compounds isolated from them. Nat Prod Commun 2018;13(10);5-1263. https://doi.org/10.1177/1934578X1801301007

40. Sharma JN, Al Omran A, Parvathy SS. Role of nitric oxide in inflammatory diseases. Inflammopharma. 2007;15:9-252. https://doi.org/10.1007/s10787-007-0013-x

41. Karthik PBRK, Priya RV, Kumar KS, Rathore RSB. Evaluation of anti-inflammatory activity of *Canthium parviflorum* by *in-vitro* method. Indian J Res Pharm Biotechnol. 2013;1(5):31-729.

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

Publisher information: Plant Science Today is published by HORIZON e-Publishing Group with support from Empirion Publishers Private Limited, Thiruvananthapuram, India.