



Chemical Compound Of Ethanol Extract From Seluang Malik (*Leea Amabilis* Veitch Ex Mast) Potential As An Anti-Diabetes

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Abstract

Background. The Bahau ethnic group in East Kalimantan has employed the roots and leaves of the *L. amabilis* plant as an anti-diabetic remedy. Thus, scientific verification of this herb is required.

Aims. The goal of this study is to examine the bioactive compounds, the anti-diabetic potential, and the chemical composition of *L. amabilis* ethanolic extracts.

Methods. Phytochemical analyses, -amylase enzyme activity assays, DPPH free radical scavenging activity tests, and GC/MS chemical compound analyses were used as experimental methodologies.

Result. The findings demonstrated the presence of flavonoids, tannins, steroids, carbohydrates, and carotenoids in the leaf extracts. Alkaloid, tannin, triterpene, and carbohydrates were present in the root extracts. Alkaloid, tannin, triterpene, and carbohydrates were present in the root extracts. The antioxidant and anti-diabetic activity of the *L. amabilis* leaves extract is higher than that of the root. A significant chemical in the leaves and roots of *L. amabilis* has been identified as endo-Borneol.

Conclusion. A substantial chemical in the leaves and roots of *L. amabilis* has been identified as endo-Borneol. Endo-Borneol from leaves of *L. amabilis* showed a chromatographic peak at R. Time of 7.327, area of 2483598, area% of 43.64%, height of 956167, and A/H of 2.60. Nevertheless, the Endo-Borneol chromatographic peak from the root of *L. amabilis* was found at R. Time of 7.605, area of 889232, area% of 100%, height of 49189, and A/H of 18.08.

Implementation. The findings of the study highlight the need for further processing of natural antioxidant and anti-diabetic compounds made from *L. amabilis* leaves.

Keywords: α -amylase enzyme; anti diabetes; antioxidant; endo-Borneol; flavonoid; *Leea amabilis*



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INTRODUCTION

Natural plants are an excellent source for drug discovery. Many metabolites from various parts of the herb have not been screened for biological activity. Seluang Malik (*Leea amabilis* Veitch ex Mast) (Family Vitaceae) has long been used as an antidiabetic agent,

Rosamah

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| 14

according to local wisdom, by the Bahau ethnic group in East Kalimantan, Indonesia. *L. amabilis* spreads in the regions of Borneo and Sarawak. It has serrations on the edges and a distinctive white patch on the surface of the leaves. Scientific validation of medicinal plants is vital due to the lack of research related to these plants. There are still many potential plants with unknown sources of antidiabetic chemical compounds, which can support efforts in optimizing the utilization of natural products and reducing dependence on pure chemical synthetic products.

According to the World Health Organization, about 75% of the world population relies on traditional medicinal systems, with plant-derived products being used prominently (Kasharusani et al., 2019). In 2015, around 10 million people were living with diabetes. Indonesia ranks 7th in the number of diabetics in the world after Mexico (Cho et al., 2010). The treatment for diabetes mellitus using herbal medicine is raising interest to avoid the side effects associated with the antidiabetic agents (Bhaladi et al., 2008). Therefore, in the care of diabetes mellitus, the traditional herbal medicines are mainly taken from plants (Nair et al., 2013).

Diabetes mellitus is associated with a reduction in antioxidant potential and an augmented formation of free radicals. The levels of antioxidant parameters are found to decrease in patients with diabetes mellitus. Therefore, numerous studies suggest that phytochemicals improve insulin sensitivity by exerting antioxidant and free radical scavenging activities. The complications of diabetes can be addressed using several phytochemical compounds such as flavonoids, lignans, and propenylphenols (Bacanli et al., 2019).

Research was conducted using in vitro studies, which show an appreciable increase in glucose uptake by yeast cells when combined with plant extracts. It was observed that the plant extracts inhibited glycosylation of haemoglobins, thereby helping to inhibit the formation of glycated end products (Nair et al., 2013).

The traditional herbal medicine obtained from plants is mainly used for the treatment of diabetes mellitus (Mukesh et al., 2013). The treatment of diabetes mellitus by conventional medicine has demonstrated good clinical practice and shows a bright future (Malviya et al., 2013).

The purpose of this research is to analyse the bioactive compounds, examine the antioxidant activity and antidiabetic potential, and evaluate the chemical compounds of crude ethanolic extracts from *L. amabilis* roots and leaves.

Preventing the oxidative stress that could lead to the pathogenesis of diabetes mellitus can be done by increasing the intake of natural antioxidants to help maintain a tolerable

antioxidant status. Due to the lack of satisfactory cures in modern medicines, the bioactive compounds of medicinal plants are used as antidiabetic, chemotherapeutic, anti-inflammatory, and antiarthritic agents (Eza et al., 2012 ; Nair et al., 2013).

Among secondary metabolites, flavonoids are the most important group of bioactive compounds. The antioxidant and antidiabetic effects of flavonoids increase by increasing the total number and configuration of hydroxyl groups on the compounds (Sarian et al., 2017).

METHOD

Plant Material and Preparation of Extracts

Plant samples were converted into powder using a blender after being dried naturally, and were measured for moisture content. The uniformity of the test sample size was achieved by sieving it using an electric shaker. Preparation of test samples was carried out by referring to the standard method (Kusuma et al., 2010). The extraction process was carried out using a maceration method in ethanol for 2 x 24 hours. The extract solution was concentrated with a rotary evaporator (EYELA-FE02116) and dried in a vacuum to obtain the dried extract. The obtained extract was weighed to calculate the yield. The extracts were stored at 4 °C in the refrigerator until it was used for testing.

Antioxidant activity test

The free radical scavenging activities of *L. amabilis* leaves and root extracts were analyzed by observing DPPH free radical absorption using a UV-5200 UV/Vis spectrophotometer at a wavelength of 517 nm.

Testing of α -amylase inhibitory activity

The activity of α -amylase inhibitory was evaluated according to the standard method for testing α -amylase inhibitory activity (Kim et al., 2010).

Phytochemical analysis

Phytochemical screening was conducted by observing the color changes in the ethanolic extract of *L. amabilis*. Phytochemical analysis was done according to (Kokata et al., 2001) and (Senthilmurugan et al., 2017) methods, to evaluate the presence of bioactive compounds, including: alkaloids, flavonoids, tannins, triterpenoids, steroids, carbohydrates, coumarin, carotenoids, and saponins.

DISCUSSION

Antioxidant activity

The inhibitory activities of ethanol extracts against DPPH free radical are shown in Fig. 1. The maximum inhibition was obtained in the extract of *L. amabilis* leaves at a concentration of 12.5 ppm with a percentage of inhibition of 83.64%. The rate of inhibition approached the percentage of free radical scavenging of ascorbic acid, which was used as a positive control, with a value of 88.97% at the same concentration. Meanwhile, the lowest percentage of free radical scavenging was detected in the extract of *L. amabilis* root at a concentration of 6.25 ppm with an inhibition value of 17.14%.

The inhibitory activity of ethanolic extract from *L. amabilis* leaves against DPPH free radicals. Leaf extract at 12.5 µg/ml concentrations gave the greatest inhibition of 83.63% (Fig. 1). The inhibition percentage approached the percentage inhibition of free radical of ascorbic acid as a positive control with a value of 88.98%. There was a correlation between the flavonoid bioactive compounds and the ability to scavenge DPPH free radicals.

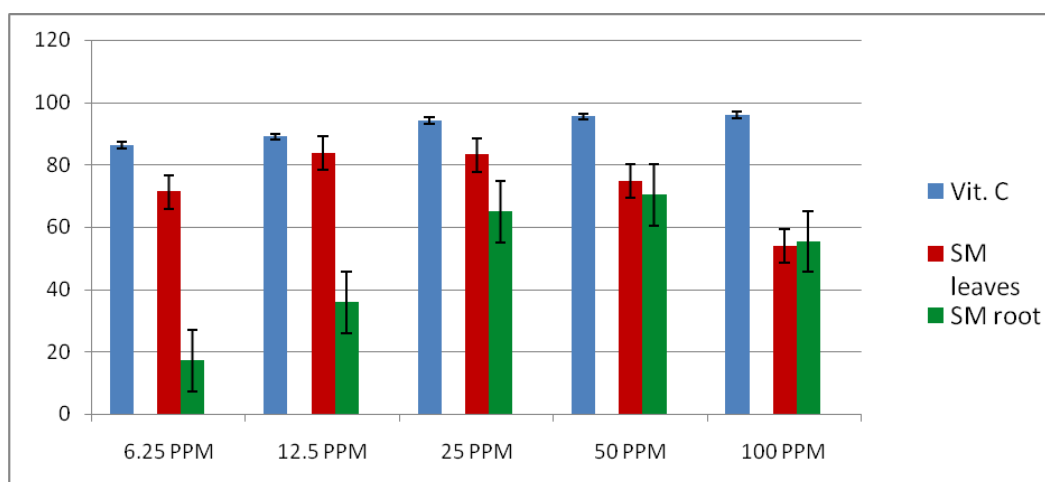


Figure 1. The free radical scavenging activity (%) of Seluang Malik (*L. amabilis*) extract

Inhibitory activity of α -amylase enzyme

Testing of the inhibitory activity of *L. amabilis* against the α -amylase enzyme was carried out using different concentrations. Based on the α -amylase inhibition assays, IC_{50} of each extract was calculated. IC_{50} is the concentration of the extract required to inhibit 50% of α -amylase activity under the assay condition.¹⁰

Samples with the lowest IC₅₀ value have the best ability to inhibit the activity of the α -amylase enzyme. The inhibition potency of crude extract against α -amylase enzyme activity is presented in Table 1. From Table 1, it appears that both extracts are potent inhibitors of the α -amylase enzyme, with the leaf extract being more active than the root extract.

Table 1. The activity of *L. amabilis* extract against α -amylase enzyme

Part of plant	Inhibition (%) by different concentration				
	50 μ g/ml	25 μ g/ml	12.5 μ g/ml	6.25 μ g/ml	IC ₅₀ μ g/ml
Leaves	99.04	79.17	52.56	42.63	9.21
Root	93.36	79.62	69.43	31.75	15.78
Quercetin					4.08

The increased efficiency of the leaf extract was also characterized by the increased presence of phenolic compounds, suggesting a relationship between its inhibitory potential on α -amylase and its content in phenolic compounds (Agnaniet et al., 2016).

Based on the results obtained from testing the ethanolic extract of leaves and roots of *L. amabilis*, it was found that the ethanolic extract of leaves had an IC₅₀ value of 9.21 μ g/ml. In contrast, the quercetin, as a positive control, had an IC₅₀ value of 4.08 μ g/ml. This proves that *L. amabilis* leaves have better inhibition potency against α -amylase enzymes with successive percentages of 99.04%, 79.17%, 52.56%, and 42.63% at concentrations of 50 μ g/ml, 25 μ g/ml, 12.5 μ g/ml, and 6.25 μ g/ml, respectively.

The presence of phytochemicals in these plants might explain these inhibitions, and the plants may contain herbal bioactive compounds (Nair et al., 2013).

Phytochemical analysis

The results of preliminary phytochemical screening of the ethanolic extract of *L. amabilis* leaves revealed the presence of flavonoids, tannins, steroids, carbohydrates, and carotenoids. However, the ethanolic extract of the root revealed the presence of alkaloids, tannin, triterpenoids, and carbohydrates. The results of the analysis of phytochemical compounds on the leaves and roots of *L. amabilis* are presented in Table 2.

Table 2. Bioactive compounds from extract of *L. amabilis*

Phytochemicals

Part of plant	Alkaloids	Flavonoid	Tannin	Triterpenoid	Steroid	Carbohydrates	Coumarin	Carotenoids	Saponin
Root	+	-	+	+	-	+	-	-	-
Leaves	-	+	+	-	+	+	-	+	-

(+) the bioactive component present in the samples, (-) the absence of the component

Table 2. Bioactive compounds from extract of *L. amabilis*

Phytochemicals	Part of plant	
	Root	Leaves
Alkaloids	+	-
Flavonoid	-	+
Tannin	+	+
Triterpenoid	+	-
Steroid	-	+
Carbohydrates	+	+
Coumarin	-	-
Carotenoids	-	+
Saponin	-	-

(+) the bioactive component present in the samples, (-) the absence of the component.

Analysis of phytochemical compounds showed that the ethanolic extract of *L. amabilis* leaves contained flavonoids and tannins. Flavonoids can protect the growth and development of animals and plants against microorganisms and pests (Masry et al., 2002). According to (Ali et al., 2017), flavonoids have anticancer, antiviral, antioxidant, antibacterial, and anti-inflammatory properties. Tannin generally possesses antioxidant, anticancer, antimutagenic, and antimicrobial properties and can help in the prevention and treatment of diseases (Yang et al., 2009). Tannin is a complex substance that has various macromolecules such as protein, starch, cellulose, and minerals (Bele et al., 2010). Flavonoids have pharmacological properties, including their capacity to act as potent antioxidants, which play an essential role in the diminishment of diabetes mellitus (Sarian et al., 2017).

Metabolites such as alkaloids, carbohydrates, carotenoids, steroids, triterpenes and their glycosides, tannins, terpenes, phenolics, and related compounds have established pharmacological bioactivity (Bandaranayake et al., 2002) ; Das et al., 2019).

Flavonoid monomers are associated with diabetes and diabetic complications. These monomers include kaempferol, myricetin, and quercetin. Many studies have shown that

flavonoids effectively inhibit α -glucosidase, reduce blood sugar levels, and protect the pancreas. The flavonoids had significant antidiabetic effects; the high level of flavonoids in the diet can reduce the incidence of diabetes (Bai et al., 2019).

Regarding their antidiabetic properties, flavonoids, as one of the polyphenolic compounds, have received the most attention among the classes of compounds. As natural polyphenolic molecules of plant origin, flavonoids possess antioxidant, anticarcinogen, and anti-inflammatory properties (Coman et al., 2012).

GC-MS analysis

Analysis of ethanolic extract from *L. amabilis* leaves was conducted using gas chromatography mass spectrometry (GC-MS) because of the volatile nature of some chemical compounds. The chromatogram of the ethanolic extract from *L. amabilis* leaves is presented in Figure 2. The chromatographic peak of Endo-Borneol from *L. amabilis* leaves was detected at R. Time of 7.327, area of 2483598, Area% of 43.64%, height of 956167, A/H of 2.60. The structure of the chemical compounds extracted from *L. amabilis* leaves can be identified by the GC-MS gas mobile phase (Table 3).

Table 3. The peak report of the constituent compounds from ethanolic extract of *L. amabilis* leaves by GC-MS

Peak	R. Time	Area	Area%	Compounds name in leaves extract
1	1.539	19216	0.34	Boric acid (H ₃ BO ₃), trimethyl ester (CAS) Trimethyl borate
2	1.749	21366	0.38	Hexane, 2,2-dimethyl- (CAS) 2,2-Dimethylhexane
3	5.047	54823	0.96	1.8 Cineole
4	6.946	239858	4.22	Camphor
5	7.327	2483598	43.64	Endo-Borneol
6	20.910	421154	7.40	Dotriacontane (CAS) n-Dotriacontane
7	24.250	211262	3.71	Tetrapentacosan
8	24.419	1230224	21.62	Tetrapentacosan
9	24.575	489968	8.61	Tetrapentacosan
10	25.852	518987	9.12	Tetrapentacosan

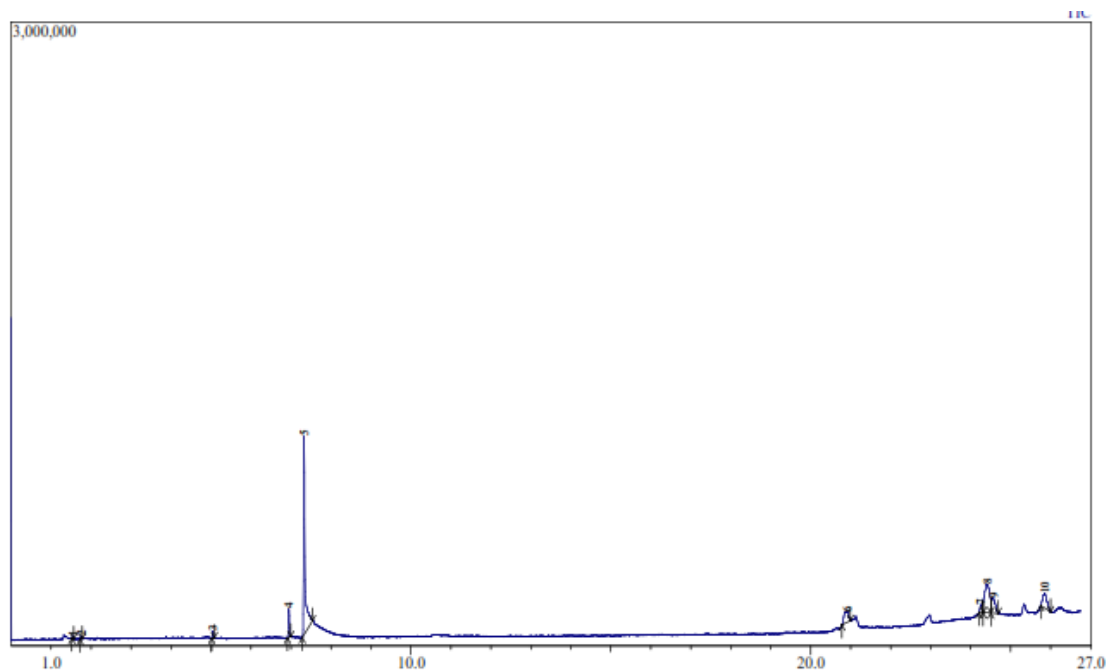
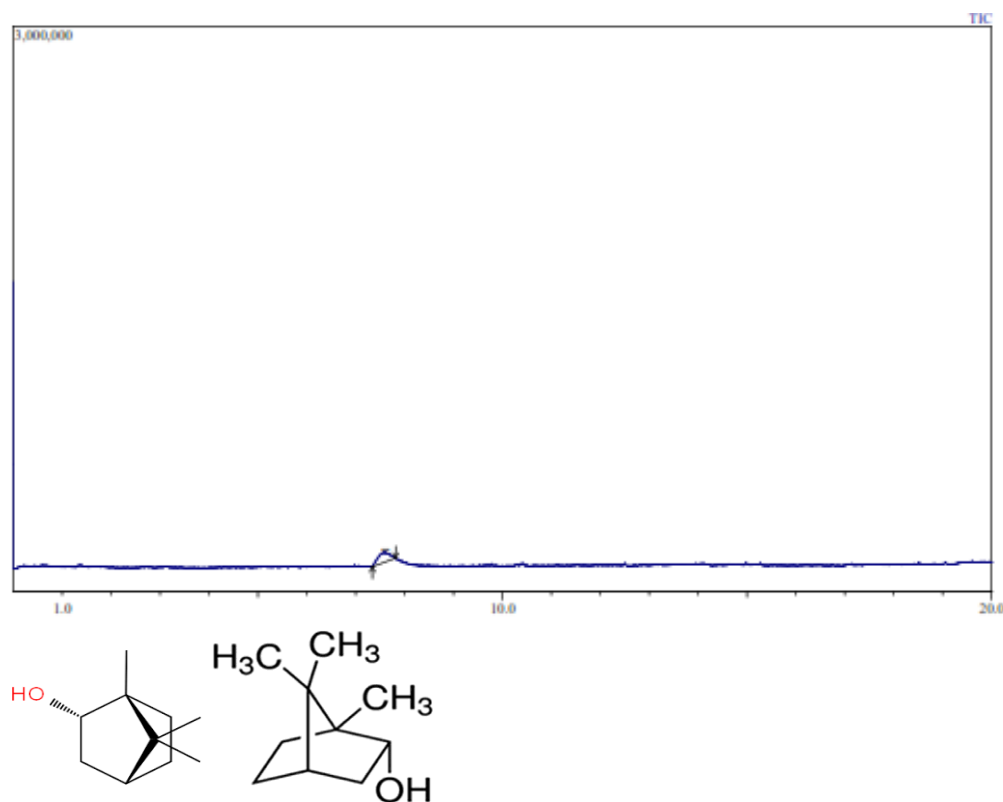


Figure 2. Chromatogram of Seluang Malik (*L. amabilis*) leaves extract

However, the chromatographic peak of Endo-Borneol from *L. amabilis* root was detected at R. Time of 7.605, area of 889232, area% % of 100%, height of 49189, A/H of 18.08. The endo-borneol compound is the only compound identified from the ethanolic extract of *L. amabilis* root, as shown in Table 4 and Figure 3. Borneol, which occurs in the essential oils of numerous medicinal plants of the families Asteraceae, Dipterocarpaceae, Lamiaceae, and Valeriaceae, is a bicyclic monoterpene alcohol (Madhuri et al., 2017). Several monoterpenes, such as carvacrol, D-limonene, citronellol, thymoquinone, thujone, safranin, myrtenol, and menthol, have shown potent antidiabetic properties (Madhuri et al., 201, while geraniol (Bakukuman et al., 2017) and carvone (Muruganathan et al., 2017) have demonstrated other beneficial effects. The terpenoid class of natural products, such as monoterpenes, is the most abundant component of essential oils and has antidiabetic effects. These compounds are biosynthesized through the melatonin pathway. They have some general antioxidant and antimicrobial effects at relatively high concentrations (Habtemarian et al., 2018).



Molecular Structure of (endo)-Borneol

Molecular formula: $C_{10}H_{18}O$

IUPAC name: 1,7,7-Trimethylbicyclo (2.2.1) heptan-2-ol

Molecular weight :154.253 g/mol

Figure 3. Chromatogram and Molecular Structure of (endo)-Borneol of (*L. amabilis*) root extract

Table 4. The elucidation of the constituent compounds from ethanolic extract of *L. amabilis* root by GC-MS

Peak	R. Time	Area	Area%	Compounds name in root extract
1	7.605	889232	100.00	endo-Borneol

Borneol plays a vital role in a wide array of therapeutic properties, such as antimicrobial, antifungal, radical scavenging, and antioxidant. The present findings are in harmony with previous studies reported by (Madhuri et al., 2017). An effective drug should have both antioxidant and antidiabetic properties, which might be beneficial in modulating diabetes. The elucidation of the constituent compounds from the ethanolic extract of *L. amabilis* leaves and root by GC-MS showed that endo-Borneol is a significant component.

However, in the ethanolic extract of *L. amabilis* leaves, some minor compounds were found, such as 1.8-cineole and camphor. In this case, endo-borneol and some compounds play a potential synergistic role in inhibiting the α -amylase enzyme activity. Therefore, the inhibition of the ethanolic extract of *L. amabilis* leaves against α -amylase enzyme was higher than that of roots. In addition, the results of the inhibition of α -amylase enzyme activities were observed in this work. They were comparable to those studied by (Madhuri et al., 2017), who found that borneol could be a potential therapeutic anti-diabetes molecule of biological relevance.

Borneol compound with model STZ-induced diabetic rats of 25 or 50 mg/kg, for 30 days led to lower blood glucose and HbA1c; increase blood insulin; restore body weight loss; increase liver glycogen level; reverse the diabetes-induced increase in the levels of TC, TGs LDL-C, VLDL-C; restore urea and ALT and AST levels; increase antioxidant status (SOD, catalase, GSH) in the liver and kidney; reduce MDA level (Madhuri et al., 2017). Borneol is widely distributed in nature as a component of essential oils. In the industry, borneol is used as a raw material for preparing perfumes, ester-making materials, and pharmacy ingredients. It serves as an antiseptic, antispasmodic, carminative, cardiac stimulant, respiratory aid, and anthelmintic (Gusmalina et al., 2014).

Persea Americana and *Piper umbellatum* leaves extract possess antidiabetic compounds classified as alkane, ester, and benzene ring compounds, identified by GC-MS (Nialeng et al., 2018). Review from work (Chen et al., 2019) demonstrated that benzopyrans and triterpenes had a stronger binding affinity with anti-diabetic target molecules as a template than reference drugs, e.g., Acarbose with α -Amylase and α -Glucosidase. The regulation of antidiabetic and antioxidant properties was revealed due to the vital role of the configuration and the total number of hydroxyl groups. The bioactivity of flavonoids, especially for antidiabetic properties, is attributed to the presence of the ketonic group and double bond in the essential structural features (Sarian et al., 2017).

CONCLUSION

The leaves of Seluang malik (*Leea amabilis*) have antioxidant activity as well as inhibitory activity against the α -amylase enzyme. As a result, the herb extracted as a natural product from *L. amabilis* extracts is a good candidate for natural antioxidant and anti-diabetic candidates. More research and analysis are required to determine the precise other active

compounds and mechanisms underlying the pharmacological role of borneol and other components that may be responsible for antidiabetic treatment.

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