IN VITRO ANTIDIABETIC ACTIVITY OF METHANOL AND ACETONE EXTRACT OF SECHIUM EDULE FRUIT

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Submitted by
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DEPARTMENT OF ZOOLOGY
BHARATA MATA COLLEGE
THRIKKAKKARA

Date:

CERTIFICATE

This is to certify that the project entitled "……

……." is a bonafide work done by……with Register No:…… during 2023-24 in partial fulfilment of the requirement for the award of the Bachelor Degree of Science in Zoology of Mahatma Gandhi University, Kottayam.

Head of the Department

Dr. Simi Joseph P

DECLARATION

I, **ANGELIN MARY (210021037714),** hereby declare that the dissertation work entitled **"IN VITRO ANTIDIABETIC ACTIVITY OF METHANOL AND ACETONE EXTRACT OF *SECHIUM EDULE* FRUIT"** submitted for the award of a Bachelor's degree in Bharata Mata College, Thrikkakara, is a partial fulfillment of the requirements. This work was done by me during the period from December 2023 to February 2024 under the supervision and guidance of Dr. Simi P Joseph , Assistant Professor, Zoology Department. I affirm that this thesis is original and has not been submitted for any degree, fellowship, or similar qualification by any other candidate to any university.

Date:

Place: Thrikkakara ANGELIN MARY

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ABBREVIATIONS

DM	Diabetes Mellitus
nm	Nanometer
mM	Millimolar mass
ml	Milliliter
pH	potential of hydrogen
g	Grams
%	Percentage
pNPG	p-nitrophenyl glucopyranoside
Na2CO3	Sodium Carbonate
µl	Microliter

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ABSTRACT

The objective of the study was to identify the antidiabetic potential of the Sechium edule fruit in methanol and acetone extract thereby adding another remarkable finding in the field of natural medicine. Due to the unpredictable and irreversible ill effects of synthetic medicine the future of medical science is more dependent on bio resources for major introduction of cost free, low side effect and more efficient natural sources of medicines. Since plants are the most dependent and valuable resources for human kind, more studies takes place in plants and their various aspects in order to introduce such safe granted medicine.. Since diabetes, rather than a lifestyle disease had now evolved into a lifestyle the number of people prone to this disorder is increasing in an unpredictable manner so introduction of natural medicine to reduce side effects on such a large community become a mandatory matter for the medical field. In the present study, the antidiabetic potential of Sechium edule or chayote fruit was evaluated .

The methanol and acetone extract of Chayote was extracted using fruit pulp and it was tested using in vitro antidiabetic assay protocol with the help of major chemicals like alpha glucosidase and pNPG. The absorbance of light was noted using the visible spectrophotometer at 405 nm. The absorbance value was noted and from this the inhibition percentage was calculated. The calculation proved that *Sechium edule* fruit has antidiabetic property and showed more inhibition in acetone extract than methanol extract.

Since the fruit is edible and are taken by many it can be consumed easily rather than medicines. It is the best way to prevent diabetes and also the best way for medicine haters.

INTRODUCTION

Diabetes mellitus is a condition in which human pancreas is unable to produce insulin or the body is resistant to the insulin it produces. Insulin is the hormone which is responsible for lowering the blood glucose. It is estimated that about 529 million people in the world is prone to diabetes. It is equally affected to men and women and children regardless of age. It is calculated that by 2050 it may increase upto 1.3 billion people (Kooti et al., 2016).

Many are the cause for diabetes like genetics, lifestyle, pregnancy etc. Having diabetes for a long time causes various health problems and disease like nerve damage, eye issues and damage of kidney and so on.

The patients with a slight variation are provided with medicines, but further on as it progress the patient must take insulin injections. There are many ill effects for these methods. The major problem is once taken, then must continue for the whole life and other underlying diseases. So for the production of more efficient with less ill effects medicine are in progress. Since nature has the best healing without adverse side effect, more discoveries in this field is in progress.

Chayote (*Sechium edule*, Cucurbitaceae) is increasingly recognized and valued as a food crop globally, despite being relatively lesser-known within the gourd family. Its distinctiveness lies in its production of single-seeded fruits and the phenomenon of vivipary. Chayote's adaptability to diverse climatic conditions makes it relatively easy to cultivate. Beyond its fruits, it offers tender shoots for vegetable greens, vines for ornamental purposes or animal fodder, and edible subterranean storage roots. With its commendable nutritional profile and versatile culinary applications due to its firm and delectable flesh texture, chayote merits wider adoption and utilization (Veigas et al., 2020).

The fruit of the chayote plant hangs downward, displaying a light green hue and a rough, pear-shaped form, typically flattened with coarse wrinkles. They measure between 10 to 20 cm in length. The texture of the fruit

can range from fleshy to fleshy-fibrous, often exhibiting longitudinal ridges or furrows. Chayote fruits come in various shapes, including globose, ovoid, sub-ovoid, and pyriform. Despite their diverse appearances, the taste of the flesh is generally mild and bland.

Phytochemical analysis of the chayote fruit, encompassing both pulp and seeds, has identified various bioactive compounds such as alkaloids, flavonoids, carotenoids, triterpenoids, saponins, phenolic acids, peroxidases, and essential minerals including potassium, calcium, phosphorus, and magnesium. Additionally, the seeds contain essential amino acids like leucine, arginine, phenylalanine, valine, lysine, isoleucine, threonine, and histidine, along with other valuable components such as total carotenes, tannins, protein, glycolipid, and phospholipid. One notable pharmacological action observed is its anti-hyperlipidaemic properties

Chayote exhibits diverse pharmacological effects:

Anti-hyperlipidaemic Properties: Flavonoids present in Chayote shoots reduce serum lipids and cholesterol levels, providing defense against atherosclerosis and fatty liver.

Anti-epileptic Activity: The ethanol extract derived from Chayote fruits exhibits anti-epileptic and CNS depressant effects in rat models, resulting in a noticeable decrease in convulsion duration induced by MES and PTZ, as well as a dose-dependent reduction in locomotor activity.

Anti-diabetic Activity: When administered orally to alloxan-induced diabetic rats, Chayote fruit extract promotes weight loss and significantly lowers blood glucose levels.

Anti-microbial Effect: Aqueous and ethanolic extracts obtained from Chayote leaves demonstrate antimicrobial activity against resistant strains of staphylococci and enterococci.

Anti-ulcer Activity: Oral administration of ethanol Chayote fruit extract exhibits notable anti-ulcer effects, particularly in alleviating aspirin-induced gastric ulceration in rats.

Hepatoprotective Properties: Oral administration of Chayote ethanolic fruit extracts at varying doses shows significant hepatoprotective activity against Tetrachloromethane-induced hepatotoxicity in rats.

Anti-hypertensive Effect : Hydroalcoholic extract derived from Chayote roots, including fractions and subfractions, exhibits anti-hypertensive effects in various models induced with angiotensin.

Anti-obesity Properties : Extracts from Chayote shoots enhance the activation of AMP-activated protein kinase (AMPK) while reducing several lipogenic-related enzymes, including sterol regulator element-binding proteins and HMG-CoA reductase proteins, crucial regulators of hepatic lipid metabolism.

Antioxidant Properties: Ethanolic extracts of Chayote leaves and aqueous seed extracts display strong inhibitory activity through β-carotene bleaching and a robust reducing effect through a linoleate model. Flavonols, particularly flavonoids, are identified as the main contributors to this antioxidant effect (Mogale et al., 2011)

This test can hopefully improve the more usage of plants than that of synthetic medicine. The medicines derived from plants can be used without much side effects and are easily available. It is proved that majority of plants features compounds called glycosides, alkaloids, terpenoids, flavanoids and carotenoids that can frequently prevent diabetes. Plants have the potential to enhance pancreatic tissue function, which can lead to decreased intestinal glucose absorption or increased insulin releases, which in turn has anti hyperglycemic effects (Aung et al., 1990)

Plants such as *Cucurbita ficifolia, C. maxima, Sechium edule, C. moschata, C. pepo, Ibervillea sonorae, Citrullus lanatus, Cucumis melo, and C. sativus* have been demonstrated to possess properties that can be beneficial in the treatment of diabetes. Research findings have indicated that these plants exhibit antidiabetic effects through various mechanisms that play a role in the intricate pathogenesis of DM. These mechanisms include hypoglycemic, antioxidant, anti-inflammatory, anti-obesity, protective effects on different

organs and cells, as well as the regulation of dyslipidemias. (Huerta-Reyes et al., 2022).

AIM AND OBJECTIVE

1. Collection and extraction of chayote fruit.
2. To check the antidiabetic property of chayote antidiabetic property of chayote fruit.
3. Estimate in vitro antidiabetic activity of methanol and acetone extract of the chayote fruit.

LITERATURE REVIEW

Malviya et al. (2010) analysed the antidiabetic potential of medicinal plants of various families in the sense that using traditional and natural indigenous remedies increases the chance for the prevention of diabetes since there is no known stable cure for diabetes mellitus. It was shown that medicinal substances derived from plants exhibited greater efficiency in preventing diabetes than oral hypoglycemic medications utilized in medical treatments. The plants may help in better development of medicines in the field of diabetes.

Hutagalung et al. (2021) analysed the effectiveness of ethanol extract, swartz fraction and juice of chayote on Pancreatic β – cells. *Sechium edule* (chayote) have various influences in antioxidant, antiapoptosis, antihyperglycemic etc. Here the ethanol extract, swartz fraction and juice of chayote was used to study its antidiabetic effect on β -cells of pancreas by means of diameter of the cells. The study proved that ethanol extract is more effective than others as it improves the diameter of the cells. The reduction of pancreatic β – cells in type 2 diabetes is caused by insulin resistance, which leads to gluco toxicity, high level of lipidotoxic fats, oxidative stress etc. This can be prevented by antihyperglycemic potential of chayote.

Shariff et al. (2023) analysed antioxidant activity, total phenolic content and nutrient composition of chayote shoot. The study was mostly aimed to identify the positive benefits of the chayote mostly of its shoot part. The shoot is considered into three parts – upper tier, middle tier, and lower tier. Based on the results, the maximum value of antioxidant activity was found in the upper tier of shoot section. This tier has high content of fats, crude proteins, and carbohydrates. The middle tier has large amount of minerals, Ca, Mg, P, Mo, Fe and Al and it also have highest value of crude ash. The lower tier has high value of total phenolic content, moisture and crude fibre.

Apriyani et al. (2020) investigated the effect of chayote extract on lowering blood pressure of post partum hypertension. A major cause of maternal mortality is postpartum hypertension. Mostly therapeutic medicines are used widely which brings adverse side effects for mother and child. The outcomes show that following a 7 days chayote extract intervention, there is a significant difference (p value < 0.05) between the mean systolic and diastolic blood pressure. There was a reduction of 21.64 mm Hg in the diastolic and 35.70 mmHg in the systolic pressure.

Olvera-Vazquez et al. (2019) studied on the neglected and underutilized cucurbit species with particular reference to chayote. Based on linguistic usage and the distribution of wild relatives the new world – more specifically, the Mesoamerican region was hypothesised to be the origin of chayote. A well known neglected and under utilized cucurbit species resource from Mesoamerican chayote has both therapeutic and high nutritional value, making it a potential source of food security. It has numerous health advantages.

Pu et al. (2021) studied origin, evolution, breeding and omics of chayote. Chayote one among the cucurbitacea family is used in many areas like food industry, cosmetics etc. It is major provider of minerals and nutrients. It has many medicinal properties. It was originated mainly from Mexico and Guatemata and it was proved by its genetic diversity concentration. Major researchers are leading in finding the vast establishment of chayote into medicinal field.

Huerta-Reyes et al. (2020) analysed the different species of the cucurbitacea family used in the treatment of diabetes mellitus in Mexico. The study was mainly on *Cucurbita ficifolia, C. Maxima, C. Moschata, C. Pepo, Ibervillea sonorae, Sechium edule, Citrullus lanatus, Cucumis melo, and C. Sativus*. It has vitamin C, starch, protein, peroxide and minerals like iron, manganese, zinc & calcium, antioxidant shows hyperglycemia effects etc. It showed various health benefits and also antioxidant and antiapoptosis properties.

Siahaan et al. (2020) studied the effect of the chayote ethanol and ethyl acetate fraction on insulin resistance and oxidative stress. In those with type 2 diabetes mellitus, oxidative stress resulted in insulin resistance and irregular insulin production. The attempt was to investigate chayotes potential on insulin resistance and oxidative stress. The study was conducted in white rat and the result proved that chayote extract worked as antioxidant.

Abdelhai et al. (2023) analysed the functional properties and potential utilization of chayotes fruit starch. The amylase concentration, functional characteristics and granule morphology of Algerian chayote fruit starch were investigated. Despite challenges with extraction, starch was extracted from chayote fruit grown in Algeria's Mediterranean environment. Due to the changes in its functional qualities and existence of residual components these challenges were different from those faced with starch derived from botanical sources. The size of the starch granules varied from 3.56 to 37.24 µm. While amylopectin accounted for 76.64% of the sample amylose made up only 20.369%.

Sibi et al. (2013) tested on the antibacterial activity of *Sechium edule* against gram negative food borne bacteria . The fruit pulp and the seed of chayote were extracted using different solvents and their antimicrobial properties were evaluated. Fruit extracts were subjected to phytochemical analysis which identified to phytochemical analysis which identified the presence of tepenoids, flavnoids, alkaloids and saponins. Chloroform and methanol extracts were the two types of extracts that showed antibacterial action against majority of gram negative bacteria .

MATERIALS AND METHOD

Preparation of plant extract

Freshly cut fruit was crushed into fruit pulp. Then 10 g of fruit pulp was soaked in 60ml of methanol and acetone respectively for overnight. The extract was filtered and both the extract were stored in refrigerator for future use.

Figure 1 :Chayote fruit

Figure 2 : Fruit pulp soaked in methanol and acetone

Figure 3 : Methanol extract

Figure 4 : Acetone extract

Chemicals used

4-Nitrophenyl-α-D-glucopyranoside (pNPG) and α-glucosidase were used. Potassium dihydrogen phosphate and dipotassium hydrogen was used for preparing buffer along with sodium chloride. Na2CO3 was used as a stopper solution.

Inhibition of alpha glucosidase

Potassium Phosphate Buffer Preparation:

Phosphate buffer with pH 6.8 was prepared. Combined 1 gram of potassium dihydrogen phosphate, 2 grams of dipotassium hydrogen phosphate, and 8.5 grams of sodium chloride in 900 ml of water. Adjust the pH to 6.8 and then add more solvent to make the total volume 1000 ml.

In a laboratory setting, 50 microliters of α-glucosidase solution with a concentration of 0.5 units per ml were combined with 50 microliters of 0.2 Molar potassium phosphate buffer at pH 6.8. This mixture was then mixed with test samples. After being kept at 37°C for 15 minutes, 100 microliters of 3 mM pNPG solution was added. The reaction continued for another 10 minutes at the same temperature, after which it was terminated with 750 microliters of 0.1 molar Na2CO3 solution. The absorption of 4-nitrophenol was measured at 405 nm in spectrophotometer. Controls were set up using reaction mixtures without the test sample and without the substrate, and the experiment was repeated three times for accuracy (Kim et al.,2011)

The percentage inhibition of α-glucosidase was calculated as follows:

Alpha glucosidase inhibition (%) = (A control – A sample)/A control ×100

Where A control is absorbance of the control

A sample is absorbance of the experimental sample

Figure 5 : Potassium Phosphate Buffer

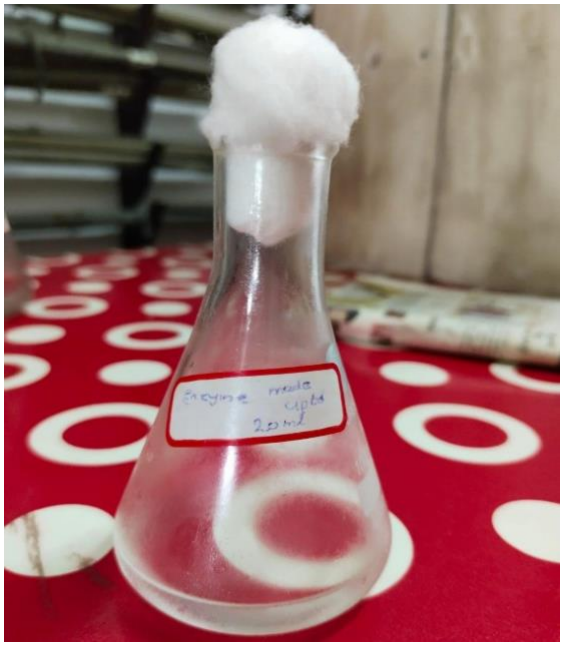

Figure 6 : Alpha glucosidase

Figure 7 : pNPG

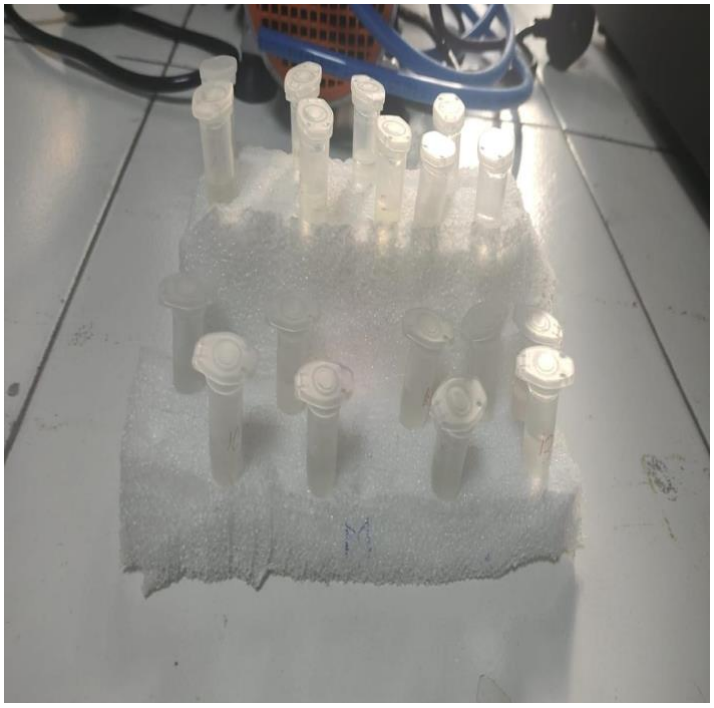

Figure 8 : Sample for measuring absorbance

Figure 9 : Spectrophotometer

RESULT AND DISCUSSION

Antidiabetic property is shown by many natural products. So the present study was based on this fact and was conducted on friut *Sechium edule.* The antidiabetic assay was performed with the help of visible spectrophotometer at 405 nm which gave the absorbance of both methanol and acetone extract, thereby facilitating in calculating the inhibition percentage which determined the inhibition of alpha glucosidase by sample at various concentrations.

The absorbance values obtained were :

Concentrations of the sample	Absorbance of methanol extract
50 µl	0.098
50 µl	0.083
50 µl	0.072
25 µl	0.120
25 µl	0.110
25 µl	0.080
15 µl	0.055
15 µl	0.051
15 µl	0.160

Concentration of the sample	Absorbance of acetone extract
50 µl	0.014
50 µl	0.021
50 µl	0.018
25 µl	0.034
25 µl	0.039
25 µl	0.029
15 µl	0.099
15 µl	0.082
15 µl	0.079

The absorbance value of control obtained was:

Methanol control = 1.812

Acetone control = 1.92

The percentage inhibition of α-glucosidase was calculated as follows :

Alpha glucosidase inhibition (%) = (A control – A sample)/A control ×100

The % inhibition : ● Methanol extract

Concentration of the sample	Average absorbance	Inhibition percentage
50 µl	0.084	95.36%
25 µl	0.103	94.31%
15 µl	0.088	95.14%

● Acetone extract

Concentration of the sample	Average absorbance	Inhibition percentage
50 µl	0.071	96.3%
25 µl	0.034	98.23%
15 µl	0.086	97.86%

This study was conducted to examine the antidiabetic activity of *Sechium edule* fruit . The present study of *Sechium edule* on antidiabetic assay proved that the fruit showed antidiabetic activity and it showed more inhibition activity in acetone extract.

It also revealed that as the absorbance of the sample decreased the inhibition percentage increased. Higher the concentration of the sample lower was the absorbance rate as a result they showed high inhibition activity. It proved that *Sechium edule* could be used as a for diabetes control.

Similar results were reported by Veigas et al. (2020) where they found that when administered orally to alloxan-induced diabetic rats, Chayote fruit extract promoted weight loss and significantly lowered blood glucose levels.

In studies in human pancreas it was proved that flavonoids were also insulin mimetic, which could stimulate glycogen synthesis, and as an insulin secretagogue, which stimulated insulin production and had a protective effect on pancreatic β cells (Hutagalung et al., 2021).

Many other studies have also obtained similar results in which ethanolic extracts of *Sechium edule* were also effective in inhibitory action. The study was conducted in visible spectrophotometer due to its availability but more specific and accurate value and result would be obtained from uv spectrophotometer.

The present study proved that the fruit *Sechium edule* has antidiabetic property and was more specific in acetone extract when compared to methanol extract.

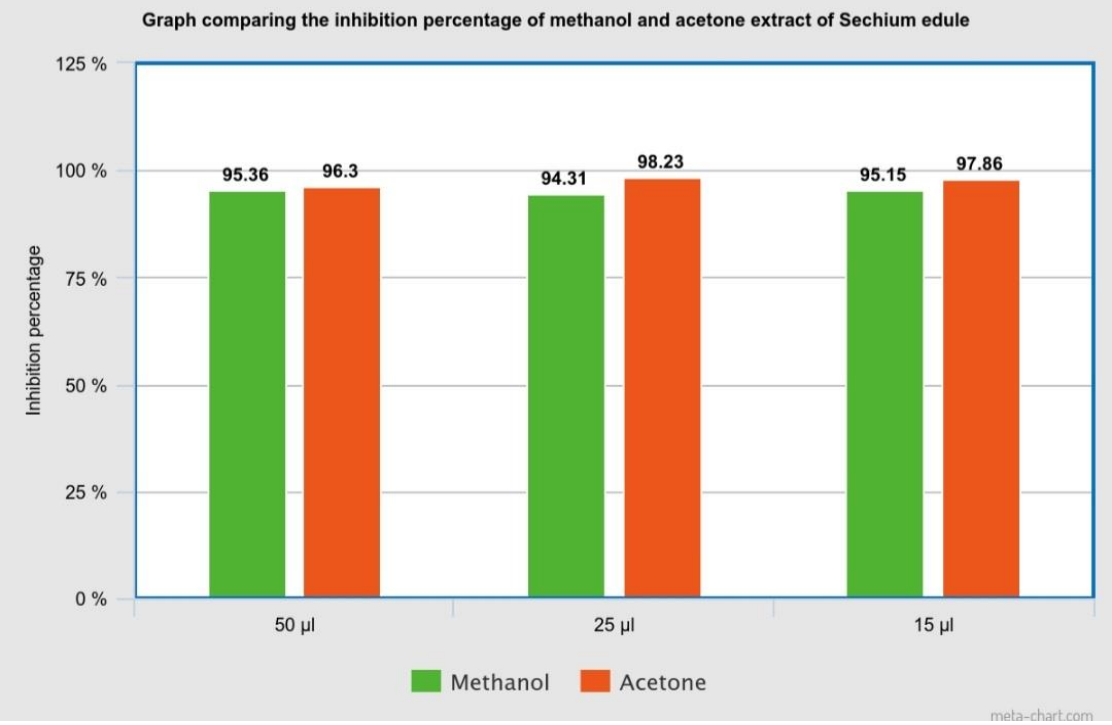

Figure 10 : Graph illustrating the inhibition percentage of methanol and acetone extract of *Sechium edule* fruit.

CONCLUSION

The present study of in-vitro antidiabetic assay was conducted in order to understand the antidiabetic potential of fruit of *Sechium edule* and to compare the potential of methanol and acetone extracts of Chayote. The major aim of the study was based on the antidiabetic effect of chayote so that it could render the hope for diabetic medicines and for the better contributions in natural derived medicine thereby reducing the side effects of synthetic medicines. The study was conducted in in-vitro antidiabetic assay protocol with the help of spectrophotometer. From the absorbance of light the inhibition percentage was calculated. The study showed that the chayote fruit do exhibits anti diabetic properties and it's acetone extract is slightly more inhibitory in nature. So including chayote in day to day diet would help to prevent the condition diabetes mellitus to some extent.

From the study it is concluded that chayote fruit has antidiabetic property and is more effective with acetone extract than that of methanol extract.

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