

Full Length Research Paper

Studies on phytochemical constituents of six Malaysian medicinal plants

Krishnaiah D.*, Devi T., Bono A. and Sarbatly R.

Chemical Engineering Program, School of Engineering and Information Technology, University of Malaysia Sabah, Locked Bag No. 2073, 88999 Kota Kinabalu, Sabah, Malaysia.

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Tannins, phlobatannins, saponins, flavonoids, terpenoids, cardiac glycosides and alkaloids distribution in six Malaysian medicinal plants, where each medicinal plant belongs to different families were examined and compared. The plants used are *Azadirachta indica*, *Centella asiatica*, *Embllica officinalis*, *Hibiscus rosa-sinensis*, *Imperata cylindrica*, and *Moringa oleifera*. Qualitative analysis carried out on each plant shows that tannins, saponins, flavonoids, terpenoids and alkaloids were present in all the plants. Phlobatannins were found to be present in *C.asiatica* and *M.oleifera* only and were absent in the rest of the plants. Cardiac glycosides were present in *A.indica*, *C.asiatica* and *I.cylindrica* and found to be absent in *E.officinalis*, *H.rosa-sinensis* and *M.oleifera*. The significance of the phytochemical constituents with the respect to the role of these plants in traditional medicine treatment is discussed.

Key words: Medicinal plants, traditional medicine, phytochemical constituents.

INTRODUCTION

The world is rich with natural and unique medicinal plants. Medicinal plants are now getting more attention than ever because they have potential of myriad benefits to society or indeed to all mankind, especially in the line of medicine and pharmacological. The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body (Akinmo-ladun et al., 2007). Some of the most important bioactive phytochemical constituents are alkaloids, essential oils, flavonoids, tannins, terpenoid, saponins, phenolic compounds and many more (Edeoga et al., 2005). These natural compounds formed the foundations of modern pre-scription drugs as we know today (Goh et al., 1995).

Phytochemical is a natural bioactive compound found in plants, such as vegetables, fruits, medicinal plants, flowers, leaves and roots that work with nutrients and fibers to act as an defense system against disease or more accurately, to protect against disease. Phytochemicals are divided into two groups, which are primary and secondary constituents; according to their functions in plant metabolism. Primary constituents comprise common su-

gars, amino acids, proteins and chlorophyll while secondary constituents consists of alkaloids, terpenoids and phenolic compounds (Krishnaiah et al., 2007) and many more such as flavonoids, tannins and so on.

Azadirachta indica, *Centella asiatica*, *Embllica officinalis*, *Hibiscus rosa-sinensis*, *Imperata cylindrica*, and *Moringa oleifera* are chosen to study because they come in abundant source, easily available, and some of them are already being utilized in traditional medicine. By studying the presence of phytochemical in these plants, the uses of these plants in traditional treatment can be explained scientifically.

MATERIALS AND METHODS

Collection of plant samples

The plant materials are brought from local markets or collected from local area. The plants are processed and analyzed.

Processing of plant samples

The leaves of the plants are properly washed in tap water and then rinsed in distilled water. The rinsed leaves are dried in an oven at a temperature of 35-40°C for 3 days. The dried leaves of each plant are pulverized, using a sterile electric blender, to obtain a powdered form. The powdered form of these plants is stored in airtight glass

*Corresponding author. E-mail: krishna@ums.edu.my. Tel: +60-88-320000. Fax: +60-88-320348.

containers, protected from sunlight until required for analysis.

Preparation of aqueous extract of plant samples

The aqueous extract of each plant sample is prepared by soaking 10 g of powdered samples in 200 ml of distilled water for 12 h. The extracts are then filtered using filter paper or Whatman filter paper.

Phytochemical analysis

Chemical tests are conducted on the aqueous extract of each plant sample and also of the powdered form of the plant samples using standard methods Edeoga et al. (2005).

Qualitative analysis on phytochemical constituents

Test for tannins

0.5 g of powdered sample of each plant is boiled in 20 ml of distilled water in a test tube and then filtered. The filtration method used here is the normal method, which includes a conical flask and filter paper. 0.1% FeCl_3 is added to the filtered samples and observed for brownish green or a blue black colouration, which shows the presence of tannins.

Test for phlobatannins

10 ml of aqueous extract of each plant sample is boiled with 1% HCl acid in a test tube or conical flask. If the sample of plant carries phlobatannins, a deposition of a red precipitate will occur and indicates the presence of phlobatannins.

Test for saponins

2 g of powdered samples of each plant is boiled together with 20 ml of distilled water in a water bath and filtered. 10 ml of the filtered sample is mixed with 5 ml of distilled water in a test tube and shaken vigorously to obtain a stable persistent froth. The frothing is then mixed with 3 drops of olive oil and observed for the formation of emulsion, which indicates the presence of saponins.

Test for flavonoids

A few drops of 1% NH_3 solution is added to the aqueous extract of each plant sample in a test tube. A yellow coloration is observed if flavonoid compounds are present.

Test for terpenoids

5 ml of aqueous extract of each plant sample is mixed with 2 ml of CHCl_3 in a test tube. 3 ml of concentrated H_2SO_4 is carefully added to the mixture to form a layer. An interface with a reddish brown coloration is formed if terpenoids constituent is present.

Test for cardiac glycosides

1 ml of concentrated H_2SO_4 is prepared in a test tube. 5 ml of aqueous extract from each plant sample is mixed with 2 ml of glacial $\text{CH}_3\text{CO}_2\text{H}$ containing 1 drop of FeCl_3 . The above mixture is carefully added to the 1 ml of concentrated H_2SO_4 so that the concentrated H_2SO_4 is underneath the mixture. If cardiac glycoside is

present in the sample, a brown ring will appear, indicating the presence of the cardiac glycoside constituent.

Quantitative analysis on phytochemical constituents

Phenols

The quantity of phenols is determined using the spectrophotometer method. The plant sample is boiled with 50 ml of $(\text{CH}_3\text{CH}_2)_2\text{O}$ for 15 min. 5 ml of the boiled sample is then pipetted into 50 ml flask, and 10 ml of distilled water is added. After the addition of distilled water, 2 ml of NH_4OH solution and 5 ml of concentrated $\text{CH}_3(\text{CH}_2)_3\text{CH}_2\text{OH}$ is added to the mixture. The sample is made up to the mark and left for 30 min to react for colour development and measured at 505 nm wavelength using a spectrophotometer.

Alkaloids

5 g of the plant sample is prepared in a beaker and 200 ml of 10% $\text{CH}_3\text{CO}_2\text{H}$ in $\text{C}_2\text{H}_5\text{OH}$ is added to the plant sample. The mixture is covered and allowed to stand for 4 h. The mixture then filtered and the extract is allowed to become concentrated in a water bath until it reaches 1/4 of the original volume. Concentrated NH_4OH is added until the precipitation is complete. The whole solution is allowed to settle and the precipitate is collected and washed with dilute NH_4OH and then filtered. The residue is alkaloid, which is then dried and weighed.

Tannins

Quantity of tannins is determined by using the spectrophotometer method. 0.5 g of plant sample is weighed into a 50 ml plastic bottle. 50 ml of distilled is added and stirred for 1 h. The sample is filtered into a 50 ml volumetric flask and made up to mark. 5 ml of the filtered sample is then pipetted out into test tube and mixed with 2 ml of 0.1 M FeCl_3 in 0.1 M HCl and 0.008 M $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$. The absorbance is measured with a spectrophotometer at 395 nm wavelength within 10 min.

Saponins

The samples were ground and 20 g of each plant sample is put into a conical flask and 100 ml of 20% $\text{C}_2\text{H}_5\text{OH}$ is added to the plant sample. The sample is heated over a hot water bath for 4 h with continuous stirring at about 55°C . The mixture is then filtered and the residue re-extracted with another 200 ml of 20% $\text{C}_2\text{H}_5\text{OH}$. The combined extracts are reduced to 40 ml over a water bath at about 90°C . The concentrated is then transferred into a 250 ml separator funnel and 20 ml of $(\text{CH}_3\text{CH}_2)_2\text{O}$ is added to the extract and shaken vigorously. The aqueous layer is recovered while the $(\text{CH}_3\text{CH}_2)_2\text{O}$ layer is discarded and the purification process is repeated. 60 ml of $n\text{-C}_4\text{H}_9\text{OH}$ is added and the combined $n\text{-C}_4\text{H}_9\text{OH}$ extracts is washed twice with 10 ml of 5% NaCl. The remaining solution is then heated in a water bath and after evaporation; the samples are dried in the oven to a constant weight.

Flavonoids

10 g of plant sample is repeatedly extracted with 100 ml of 80% aqueous methanol at room temperature. The whole solution is then filtered through filter paper and the filtrate is later on transferred into a water bath and solution is evaporated into dryness. The sample is then weighed until a constant weight.

Table 1. Qualitative analysis on phytochemical constituents.

Plants	Tannins	Phlobatannins	Saponins	Flavonoids	Terpenoids	Cardiac glycosides	Alkaloids
<i>A.indica</i>	+	-	+	+	+	+	+
<i>C.asiatica</i>	+	+	+	+	+	+	+
<i>E.officinalis</i>	+	-	+	+	+	-	+
<i>H.rosa-sinensis</i>	+	-	+	+	+	-	+
<i>I.cylindrica</i>	+	-	+	+	+	+	+
<i>M.oleifera</i>	+	+	+	+	+	-	+

Presence of phytochemical constituents: +; Absence of phytochemical constituents: -.

Table 2. Quantitative analysis of phytochemical constituents (%).

Plants	Alkaloids	Tannins	Saponins	Flavonoids	Phenols
<i>A.indica</i>	0.52±0.12	9.1±0.20	2.1±0.13	0.62±0.10	0.024±0.13
<i>C.asiatica</i>	0.31±0.06	10.3±0.15	2.2±0.11	0.52±0.20	0.719±0.23
<i>E.officinalis</i>	0.24±0.03	11.2±0.16	1.1±0.05	0.55±0.13	0.037±0.19
<i>H.rosa-sinensis</i>	0.51±0.16	8.5±0.22	2.0±0.08	0.40±0.15	0.680±0.11
<i>I.cylindrica</i>	0.45±0.18	9.3±0.11	1.4±0.02	0.32±0.16	0.05±0.25
<i>M.oleifera</i>	0.36±0.07	9.2±0.26	2.3±0.04	0.51±0.18	0.08±0.17

RESULTS AND DISCUSSION

Qualitative analysis

Qualitative analysis carried out on each plant showed the presence of phytochemical constituents and the results are summarized in Table 1. It shows that tannins, saponins, flavonoids, terpenoids and alkaloids were present in all the plants. Phlobatannins were found to be present in *C.asiatica* and *M.oleifera* only and were absent in the rest of the plants. Cardiac glycosides were present in *A.indica*, *C.asiatica* and *I.cylindrica* and found to be absent in *E.officinalis*, *H.rosa-sinensis* and *M.oleifera*.

Quantitative analysis

The results of quantitative analysis on five major groups of phytochemical constituents in the medicinal plants is summarized and shown in Table 2. *A.indica* and *H.rosa-sinensis* have the highest yield of alkaloid, which is 0.5 g, followed by *I.cylindrica* which is 0.4 g. *C.asiatica* and *M.oleifera* contain 0.3 g of alkaloid while *E. officinalis* has the lowest yield of alkaloids, which is 0.2 g.

From the Table 2, *E.officinalis* showed the highest concentration of tannins, followed by *C. asiatica*, *I. cylindrica*, *M.oleifera* and *A. indica*. *H. rosa-sinensis* has the lowest concentration of tannins, whereas the saponins quantity in the plants, *A. indica*, *C. asiatica*, *H. rosa-sinensis* and *M.oleifera* yielded similar quantity of saponins, which are 2.1, 2.2, 2.0 and 2.3 percentages respectively. *E. officinalis* and *I. cylindrica* have the least yield of saponins. *A.indica* produced the highest yield of flavonoid, which is 0.62%, closely followed by *C. asiatica* and *E. officinalis*.

M. oleifera, *H. rosa-sinensis* and *I. cylindrica* produced the least amount of saponins. In respect of phenols *C. asiatica* and *H. rosa-sinensis* have the highest quantity of phenols. *A. indica*, *E. officinalis*, *I. cylindrica* and *M. oleifera* have quantity of phenols in the same range.

***Azadirachta indica*: effect of terpenoids, saponins, flavonoids, tannins, alkaloids**

A.indica leaves are used to treat chickenpox by directly applying to the skin in a paste form or by bathing in water with *A. indica* leaves. It has been a traditional practice since ancient time to use *A. indica* leaves to prevent further spreading of the virus, to alleviate the external symptoms of itching and to minimize scars (Conrick, 2007). From the analysis, it is known that *A.indica* is a source of terpenoid, which plays an important role in wound and scar healing (Hawkins and Ehrlich, 2006). *A.indica* leaf juice also is used for treatment of skin disease (Sotheeswaran et al., 1998). Besides, *A.indica* is as a source of pesticide and insecticide because of the presence of bioactive component azadirachtin. It has been an age-old practice in India to mix dried neem leaves with grains meant for storage for protection against insect pests. Azadirachtin is a type of terpenoid or more specifically is a highly oxidized tetranortriterpenoid. Azadirachtin is a natural insecticide and acts mainly as an antifeedant and growth disruptor, and possesses considerable toxicity toward insects (Khalid et al., 1989). The presence of azadirachtin in *A. indica* is the reason why *A.indica* is used to remove head lice in human hair (Conrick, 2007). Oil from *A. indica* seeds and *A. indica* leaves has been traditio-

nally used for diabetes, which has been confirmed in animal studies. From the research on laboratory rats, it was reported that their insulin requirements were reduced by between 30 and 50% (Dixit, 1986). This can be explained by the presence of terpenoids, as reduces complications associated with diabetes and lowers sugar level in blood (Hawkins and Ehrlich, 2006)

A. indica leaves is also used to treat hyperglycaemia (Sotheeswaran et al., 1998), and saponins have been found to be potentially useful for the treatment of hyperglycaemia (Olaleye, 2007; Malinow et al., 1977). The presence of saponins in Table 1, *A. indica* is also another reason why the leaves are used traditionally to cleanse and purify blood because one of saponins medicinal uses is as a gentle blood cleanser (Kenner and Requena, 1996). *A. indica* leaves also prevent damage caused by free radicals in the body by neutralizing them. This can be explained by the presence of flavonoids in *A. indica* leaves, as flavonoids are known to act as antioxidant. Antioxidants neutralize highly unstable and extremely reactive molecules, called free radicals, which attack the cells of human body every day (Stauth, 2007). Free radical damage is believed to contribute to a variety of health problems, including cancer, heart disease and aging (Stauth, 2007).

Young twigs of *A. indica* are used as toothbrush since ancient time in India, Bangladesh and Pakistan (Chellaiyah et al., 2006). Apart from that, *A. indica* oil also is a very popular as a traditional dentifrice. The oil from the seeds has been found to be anti-inflammatory and aids healing in gingivitis. This can be explained due to the presence of saponins and flavonoids (Table 2), as both constituent show anti-inflammatory properties (Kenner and Requena, 1996).

The presence of alkaloids and saponins (Table 2) in the leaves of *A. indica* explains why the leaves of *A. indica* used for hypertension treatment (Akinpelu and Onakoya, 2006; Raffauf, 1996, Olaleye, 2007; Malinow et al., 1977). From research, chewing of 8-10 neem leaves early in the morning for twenty four days protects the body from diseases like diabetes and hypertension (Conrick, 2007). *A. indica* is used to treat dysentery and other intestinal disorder (Akinpelu and Onakoya, 2006). The presence of tannins (Table 1) also aids in wound healing (Okwu and Josiah, 2006).

***Centella asiatica*: effect of terpenoids, flavonoids, tannins**

C. asiatica is very rich in terpenoids (Table 1), a compound which plays a very active role in wound healing (Sotheeswaran et al., 1998). From clinical studies, it is shown that terpenoids strengthen the skin, increase the concentration of antioxidants in wounds, and restore inflamed tissues by increasing blood supply. Because of these properties, *C. asiatica* has been used widely in herbal medicine for burns, psoriasis, prevention of scar for-

mation following surgery, recovery from an episiotomy following vaginal delivery of a newborn, and treatment of external fistulas (Hawkins and Ehrlich, 2006). Terpenoid also is a heart-friendly phytochemical constituent and this may be the reason why *C. asiatica* is very popular among patients with high blood pressure and diabetes because *C. asiatica* helps to reduce diastolic blood pressure and lowers the sugar level in blood (Hawkins and Ehrlich, 2006). Traditionally, *C. asiatica* is consumed as an herbal tea or the leaves are eaten fresh. The presence of terpenoid is also another reason why *C. asiatica* is used as a rejuvenating agent because it has been found to be a very useful remedy for anti-aging and overall beauty enhancement (Ramlan et al., 2003).

The presence of flavonoids and tannins (Table 2) explains the reason why the leaves of *C. asiatica* are used for the treatment of diarrhoea. The study done at Children's Hospital and Research Center Oakland, in collaboration with scientists at Heinrich Heine University in Germany, has shown that epicatechin, quercetin and luteolin, which are type of flavonoids can inhibit the development of fluids that result in diarrhoea by targeting the intestinal cystic fibrosis transmembrane conductance regulator Cl⁻ transport inhibiting cAMP-stimulated Cl⁻ secretion in the intestine (Schuier et al., 2005).

Cardiac glycosides were found to be present in *C. asiatica*, a compound that has been shown to aid in treatment for congestive heart failure and cardiac arrhythmia. This is another reason why this plant is widely used in traditional medicine. Cardiac glycosides work by inhibiting the Na⁺ /K⁺ pump. This causes an increase in the level of sodium ions in the myocytes, which then leads to a rise in the level of calcium ions. This inhibition increases the amount of Ca²⁺ ions available for contraction of the heart muscle, improves cardiac output and reduces distention of the heart.

***Embllica officinalis*: Effect of flavonoids, tannins, phenol, terpenoids, saponins and terpenoids**

From the analysis, *E. officinalis* leaves are very rich in tannins and flavonoids (Table 2). The presence of flavonoids explains the reason why *E. officinalis* leaf infusion with fenugreek seeds is used for treatment of chronic diarrhoea (Treadway, 1994) as flavonoids is effective against diarrhoea (Schuier et al., 2005). *E. officinalis* fruit has tannins as its main component and very much astringent in nature and tannins has high potential treating intestinal disorders such as diarrhoea and dysentery (Akinpelu and Onakoya, 2006).

The presence of flavonoids in the leaves and fruits of *E. officinalis* enables them to be used as an antioxidant. Experiments conducted at the Niwa Institute of Immunology in Japan have shown *E. officinalis* to be a potent scavenger of free radicals (Dweck and Mitchell, 2002).

E. officinalis is also believed to be an aphrodisiac and is considered to be one of the strongest rejuvenate herbs in

Ayurveda medicine. It is the primary ingredient used in one of the renowned Ayurveda herbal formula, called *Chayavanprasha* which has great respect as a sexual vitality tonic (Dweck and Mitchell, 2002). This may be contributed by the presence of phenolic compounds (Table 2), which acts as stimulating agent (Kenner and Requena, 1996). *E. officinalis* is also used to detoxify blood from chemicals and harmful toxic and presence of phenols may be the reason why *E. officinalis* is used in such a way because phenols act as a detoxifying agent.

Decoctions of the leaves and seeds of *E. officinalis* are used in the treatment of diabetes (Treadway, 1994) and this may be due to the presence of terpenoids (Table 1) in *E. officinalis* (Hawkins and Ehrlich, 2006). *E. officinalis* also has been a popular use for anti-inflammatory and antipyretic treatment in traditional medicine. The fresh fruit of *E. officinalis* is used in Turkistan in inflammations of the lungs (Dweck and Mitchell, 2002). Saponins (Table 2) and flavonoids have anti-inflammatory properties and this may be the obvious cause why *E. officinalis* has been used for anti-inflammatory treatment (Kenner and Requena, 1996). The juice or fruit extract of *E. officinalis* is mixed with honey and is a useful remedy for painful respiration such as dyspnoea and oligopnoea. Terpenoids (Table 1) can be the reason why *E. officinalis* is used for respiratory treatment because one of terpenoids medicinal uses is it improves lung functions (Hawkins and Ehrlich, 2006).

***Hibiscus rosa-sinensis*: Effect of flavonoids, saponins tannins, terpenoids**

The flowers and leaves of *H.rosa-sinensis* contain substantial quantities of flavonoids (Table 1) which are associated with antioxidant, fever-reducing (antipyretic), pain-relieving (analgesic) and spasm-inhibiting (spasmodic) activities. The decoction of the leaves is used in the treatment of fevers (Chopra et al., 1986) and the flower has soothing properties which are used to relieve menstrual cramps and relax spasms and general cramping. Presence of flavonoids in the leaves of *H. rosa-sinensis* is another reason why it is used to treat inflammations (Sotheeswaran et al., 1998).

H.rosa-sinensis also reduces blood pressure and cholesterol level in blood. This is due to the existence of saponins (Table 1) in *H.rosa-sinensis*. Saponins bind to cholesterol to form insoluble complexes and excreted via the bile. This prevents cholesterol reabsorption and results in a reduction of serum cholesterol. Saponins have been found to be potentially useful for the treatment of hypercholesterolemia which suggests that saponins might be acting by interfering with intestinal absorption of cholesterol (Olaleye, 2007; Malinow et al., 1977).

H. rosa-sinensis also aids in wound healing (Nayak et al., 2007) and this can be explained due to the existence of tannins (Table 2) and terpenoids (Table 1) which plays important role in promoting wound healing (Okwu and

Josiah, 2006; Hawkins and Ehrlich, 2006). The presence of terpenoids also contributes to the reason why *H.rosa-sinensis* also is used to soothe irritated tissues and the mucous membranes that line the respiratory tract, which eases hacking coughs and other respiratory ailments (Sotheeswaran et al., 1998).

***Imperata cylindrical*: Effect of tannins, saponins and alkaloids**

I. cylindrical has been employed in traditional medicine for the treatment of dysentery and urinary tract infection (Goh et al., 1995). This is due to the existence of tannins (Table 1) and because some of the medicinal uses of tannins and used to prevent urinary tract infection and intestinal disorders such as dysentery and diarrhoea (Okwu and Josiah, 2006).

The presence of alkaloids and saponins (Table 2) in the leaves of *I. cylindrical* explains why the leaves of *I. cylindrical* used for hypertension treatment (Akinpelu and Onakoya, 2006; Raffauf, 1996; Olaleye, 2007; Malinow et al., 1977). One of *I. cylindrical* ethno pharmacological uses is as antihypertensive as it exhibits the properties (Goh et al., 1995). Besides, *I. cylindrical* also exhibits anti-inflammatory properties due to the presence of saponins and flavonoids (Kenner and Requena, 1996).

***Moringa oleifera*: Effect of tannins, terpenoids, saponins and alkaloids**

Leaves of *M. oleifera*, which are rich in tannins (Table 1), are used for urinary tract infection, diarrhoea and dysentery (Fahey, 2005). The leaves and drumstick pods of *M. oleifera* are used for diabetes, hypoglycaemia and hypertension treatment. The presence of terpenoids and saponins (Table 1) explains why *M.oleifera* is used for diabetes treatment (Fahey, 2005), because both constituents ethno pharmacological uses are to treat diabetes and hyperglycaemia, a disorder often associated with diabetes (Hawkins and Ehrlich, 2006; Olaleye, 2007; Malinow et al., 1977).

The presence of alkaloids (Table 2) in *M.oleifera* together with saponins (Table 2) is the reason why *M.oleifera* is used to treat hypertension (Fahey, 2005) because saponins prevent the excessive intestinal absorption of this cholesterol and thus reduce the risk of cardiovascular diseases such as hypertension (Akinpelu and Onakoya, 2006; Raffauf, 1996; Olaleye, 2007; Malinow et al., 1977).

The oil from the seeds is applied externally for skin diseases (Fahey, 2005) and this due to the presence of terpenoids (Table 1) as terpenoids strengthen the skin, increase the concentration of antioxidants in wounds, and restore inflamed tissues by increasing blood supply (Hawkins and Ehrlich, 2006).

Conclusion

This research work has revealed further potentials of

these six plants in the area of pharmacology as potential source of useful drugs. This study therefore has provided some biochemical basis for ethno pharmacological uses of these plants in the treatment and prevention of various diseases and disorders. The phytochemical screening on qualitative and quantitative analysis shows that the leaves of the *A.indica*, *C.asiatica*, *E.officinalis*, *H.rosa-sinensis*, *I.cylindrica*, and *M.oleifera* are rich in alkaloids, tannins, saponins, terpenoids and flavonoids, which are popular phytochemical constituents.

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