Review

Clausena excavata Burm. f. (Rutaceae): A review of its traditional uses, pharmacological and phytochemical properties

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The present review describes the morphological, traditional, phytochemical and pharmacological aspects of *Clausena excavata* (Burm. f., Rutaceae). The plant grows wild in the tropical and subtropical parts of Asia and represents one of the richest sources of medicinal natural products. It is intensively used currently in traditional medicine for treatment of different illnesses. One part or a mixture of two or more parts of the plant in the form of powder or liquid doze is usually given to the patient. Phytochemically, many pure compounds from several secondary metabolite groups of *C. excavata* have been isolated from different parts of the plant including leaves, stems, stem barks, roots, root barks, rhizomes and flowers. Their chemical structures however, have been elucidated using several spectroscopic techniques. Pharmacologically, many secondary metabolites including alkaloids and coumarins have been tested for their biological activities. Anticancer, immunomodulatory, anti-HIV-1, antioxidant, antibacterialial, insecticidal, antifungal, antinociceptive, anti-malarial and antiplatelet activities of this plant have been detailed here. This paper enumerates an overview of its phytochemical and pharmacological properties, which may provide assistance to researchers to determine further, the efficacy and potency of *C. excavata* as medicinal plant.

Key words: Folklore medicine, secondary metabolites, coumarins, bioactivities, anticancer and phytomedicine.

INTRODUCTION

The discovery and identification of biologically active secondary metabolites from new promising sources, particularly of plant origin, is one of the most effective ways in which the study in medicinal plants had progressed clearly (Lewis and Elvin-Lewis, 1995). In this aspect, *Clausena excavate* (Figure 1 a, b) has since

been able to provide natural products of interest in the field of pharmacology (Blumenthal et al., 2000). *C. excavata* Burm. f. (Rutaceae) is a wild shrub, belonging to the Rutaceae family (Burkill, 1935). Clausena is a genus of about fourteen species of evergreen trees, occurring mostly in India and tropical Asia (Shier, 1983). One of the most interesting features of *C. excavata* species is its availability in several parts of Asia. The plant is easy to grow, free of pests and diseases as well as withstanding heavy pruning (Swarbrick, 1997). The focus of this review is to report the traditional uses,

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Figure 1. *Clausena excavata* Burm. f. (Rutaceae). (a) Appearance of the overall plant, (b) appearance of leaves and fruits. Source: http://www.google.com.my/search?q=images+of+clausena+excavata.

chemical constituents and biological activities of *C. excavata.* The present review paper includes phytochemistry and pharmacology of most chemical compounds (secondary metabolites) known to the plant. This report provides essential guidance for future research of the plant particularly in plants chemistry and ethnopharmacology, which could provide accessible information in its benefits for treatment.

Occurrence

C. excavata is a shrub with strong and rather objectionable smell, found from the Himalayas and China to and throughout Peninsula Malaysia (Manosroi et al., 2005). It exists as a bush growing wild and cultivated in India, South China and Southeast Asia (Blasco, 1983). In Malaysia it's locally known as "Cherek hitam", "Chemama" and "Kemantu hitam" (Descola and PĂ_ilsson, 1996). In Thailand it is known as "San Soak" (Khare, 2007).

Morphology

It is a slender tree of 10 m height. The leaves are pinnate, 60 cm long, with 10 to 15 pairs of dark green narrowly oval oblique leaflets, 3.5 to 7 cm long with pointed tips. The leaflets have a characteristic curry-like smell when crushed. Small white flowers occur in terminal clusters, followed by translucent pink berries 7 to 10 mm across, each containing 1 to 2 seeds (Santisuk, 1988). *C. excavata* has striking hourglass-shaped gynophores, which is completely glabrous. The flower composes of four membranes and they are shortly pedicel, 1/6 inches (4 mm) diameter. The fruits are 3/4 inches and the twigs are finely hairy (Swarbrick, 1997).

Traditional uses

For its traditional uses, Tamils introduced it as a potherb (Ali et al., 2000); and amongst the Malays, it's a plant of some medicinal importance (Ridley, 1925). This plant is used traditionally in the treatment of abdominal pain, snakebite and as a detoxification agent (Ridley, 1922). The pounded root is used as a poultice for sores including ulceration of the nose and the leaves also used as a poultice. A decoction of the roots is drunk for bowel-complaints, chiefly colic (Ali et al., 2000).

The plant is found to be useful in the State of Kelantan Malaysia for yaws (Gimlette and Thomson, 1939). The flowers and leaves may be boiled and the decoction taken for colic and a decoction of leaves is also given after child birth (Grieve and Scora, 1980). The leaves of this plant are used to cure cold, abdominal pain, malaria and dysentery (Wiart et al., 2004). Decayed teeth can be treated using the drayed and powdered rootstock, whereas its stem is given in colic with or without diarrhea (Yoshida, 1996). The expressed juice of the plant is used in Java for coughs, as vermifuge (Sharma, 1998) and timber is used for handles of axes.

PHYTOCHEMISTRY OF CLAUSENA EXCAVATA

Relatively, considerable work has been done on the phytochemistry of *C. excavata* in recent years, with many previously unidentified secondary metabolites now currently being reported by group of phytochemists

(Kongkathip et al., 2010). A large number of secondary metabolites, mainly alkaloids, coumarins and few limonoids have been isolated from different parts of this plant, using different techniques of extraction and purification during the last 20 years (Zhi, 2006). The structures of these compounds have also been elucidated using different spectroscopic methods. Details are provided here, as reported in this review.

Alkaloids

C. excavata is especially rich in carbazole alkaloids, which have been extracted from its different parts (Peh, 2001). An investigation on the acetone extract of the root bark of this plant yielded ten new carbazole alkaloids, namely clausine-M, -N, -O, -P, -Q, -R, -S, -U, -V and clausenatine-A, together with 39 known compounds (Xin et al., 2008). Work by (Zhang and Lin, 2000) on the stem and root bark of *C. excavata* afforded a new biscarbazole alkaloid known as Clausemine-A (Taufig et al., 2007). Singh and Bodiwala (2010) undertook the investigation on the ethyl acetate extract of the stem bark of the Malaysian C. excavata, which gave a new carbazole alkaloid, 3-carbomethoxy-2-hydroxy-7-methoxycarbazole, commonly known as Clausine-TY, together with two known carbazole alkaloids, Clausine-H and Clausine-B, while studies by Sunthitikawinsakul et al. (2003) on the rhizomes and roots of C. excavata provided six known carbazole derivatives, 3-formylcarbazole, mukonal, 3methoxycarbonylcarbazole, murrayanine, 2-hydroxy-3formyl-7-methoxycarbazole and clauszoline J. In addition, extraction process on the stems of C. excavata led to the isolation of a new carbazole alkaloid, sansoakamine, together with 11 known compounds (Sripisut and Laphookhieo, 2007).

Further work by Potterat et al. (2005) on the stems and leaves of C. excavata has yielded a new carbazole alkaloid, named clausine Z. In addition, investigations on C. excavata have produced 2 new carbazole alkaloids, clausenol and clausenine (Ito et al., 1996), whilst a new alkaloid (Cui et al., 2002) and 4 new carbazole alkaloids, clausenamine D, E, F, and G with a highly potential inhibitory effect against an antitumor promoting activity have been identified by Ito et al. (2000). Phytochemical studies on the stem bark of C. excavata initiated by Wu et al. (1993) have resulted in the isolation of two new 4prenylcarbazole alkaloids, clausene-D and -F. In addition, Puatanachokchai et al. (1998) reported seven more carbazole alkaloids, named clauszoline-A, B, C, D, E, F, and -G, isolated from the stem bark of C. excavata collected from Singapore. Further work done by Toyota and Ihara (1998) on the stem bark and root bark of C. excavata has yielded a binary alkaloid, clausenamine-A and carbazole-pyranocoumarin dimmer, clauzomarine-A, respectively. Otherwise, another investigation again by Kumar et al. (2008) on the roots, the stem bark and the

leaves of *C. excavata* grown in a greenhouse at Fruit Tree Research Station, Shizuoka, Japan afforded six new carbazole alkaloids named clauszoline-H, -I and –J from the roots; and clauszoline-K and –L from the stem bark and clauszoline-M from the leaves were isolated together with several known carbazoles. A new carbazole alkaloid, sansoakamine, together with 11 known compounds, was isolated from the stems of *C. excavata*. All structures were elucidated by spectroscopic methods. Compound 7 showed moderate anti-malarial activity against Plasmodium falciparum with a MIC value of 6.74 µg/ml (Sripisut and Laphookhieo, 2010).

Coumarins

The plant is also rich of coumarins and a lot of phytochemical screening has been done concerning the isolation of several coumarins (Phuwapraisirisan et al., 2006). He et al. (2000) reported that a new O-terpenoidal coumarin named excavacoumarin A and a known one were isolated from the leaves of *C. excavata* Burm, f. (Rutaceae) collected in Xishuangbanna, Yunnan. The structure of 1 was elucidated by spectroscopic analysis. Chemical investigation on the fruits and stems of C. excavata led to the isolation and identification of a new coumarin, namely clausenaexcavin (Laphookhieo et al., 2009). A new dimeric coumarin, diseselin B, and three new phenylpropanoids, lenisin A-C, together with eight known O-terpenoidal coumarins, were isolated from the aerial part of Clausena lenis. Lenisin A was also isolated from the aerial part of C. excavata. The structures were elucidated on the basis of 1D- and 2D-NMR experiments (HMQC, HMBC, and ¹H,¹H-COSY) (He et al., 2006). Moreover, a study of the chemical constituents of the leaves of C. excavata cultivated in a greenhouse led to the isolation and identification of 10 new furanonecoumarins named clauslactones A, B, C, D, E, F, G, H, I, and J, together with a known carbazole, clauszoline M, and a coumarin, umbelliferone (Wu and Furukawa, 1982). (Takemura et al., 2004) reported a new dimeric coumarin, diseselin B, and 3 new phenylpropanoids, lenisin A-C, together with 8 known O-terpenoidal coumarins, isolated from the aerial part of C. excavata. It is also reported that Clausenidin, O-methylmukonal, 3formyl-2,7-dimethoxycarbazole, and clauszoline-J, were isolated from the rhizomes and roots of C. excavata (Kongkathip et al., 2010).

In another study by Hong et al. (2000) two new Oterpenoidal coumarins named excavacoumarin A, B, and a known one were isolated from the leaves of *C. excavata* collected in Xishuangbanna, Yunnan. Phytochemical investigation on the aerial part of *C. excavata* collected in Xishuangbanna, Yunnan by Hongping et al. (2000) led to the isolation of six new Oterpenoidal coumarins, named excavacoumarins B, C, D, E, F and G. Xin et al. (2008) reported three new coumarins containing a C₁₀ terpenoid side chain, clauslactones R, S and T, together with 14 known coumarins, were isolated from the leaves and stems of C. excavata. In addition to a known alkaloid, some limonoids and coumarins, the new coumarins, excavatins A-M have been isolated from C. excavate (Thuy et al., 1999). Moreover, four new furanone-coumarins, clauslactones-N, -O, -P and -Q were isolated from the leaves and twigs of C. excavata (Takemura et al., 2000). Furthermore, investigation carried out by Wu and Furukawa (1982) vielded a new carbazole alkaloid, clausine-L together with 11 known compounds from the leaves of the plant species. A study of the chemical constituents of the leaves of C. excavata cultivated in a greenhouse by Ito et al. (2000) led to the isolation and identification of 10 new furanone-coumarins named clauslactones A, B, C, D, E, F, G, H, I, and J, together with a known carbazole, clauszoline M, and a coumarin, umbelliferone. The new coumarins contain a C₁₀ terpenoid side chain with a furanone (y-lactone) moiety. Further, in clauslactones A–D, the terpenoid side chain was shown to be linked to the 7,8-dioxygenated coumarin skeleton through a 1,4dioxane ring system. This is the first example of coumarins with these structural characteristics in nature (Wan, 2005).

Limonoids

Few limonoids have been isolated from this plant compared to the numbers of alkaloids and coumarins (Yamamoto and Gaynor, 2001). Sunthitikawinsakul et al. (2003) have reported for the first time a limonoid, named, clausenolide-1-ethylether isolated from the crude ethanol extract of the rhizomes and the roots C. excavata. The stem bark of C. excavata collected from Jabi, Kedah, Malaysia yielded a limonoid, named clausenarin (Sharif and Waznah, 2009). The stem bark of C. excavata collected from Jabi and Pendang, Kedah, Malaysia in December 2006 yielded a new limonoid, clausenolide-1metylether (Cordell et al., 1991). This was obtained from the methanol extract together with a known limonoid, clausenarin. In addition to the known alkaloid, few other limonoids and coumarins, particularly the new coumarins excavatins A-M have been isolated from C. excavata (Vlietinck and Vanden Berghe, 1991).

PHARMACOLOGICAL ASPECTS

Phytochemical studies on *C. excavata* have led to the isolation of a large number of secondary metabolites of different chemical groups. It was found that most of the identified compounds have exerted different biological activities (Kongkathip and Kongkathip, 2009). *C. excavata* showed diverse therapeutic activities which included anticancer, insecticidal, antifungal, antiplatelet,

antiplasmodial, antinociceptive, immunomodulatory, antimycobacterial, and anti-HIV-1 activities (Balandrin et al., 1993).

Anticancers

Cancer remains as one of the most leading cause of death worldwide. For the past decade, there have been a tremendous number of studies and investigations conducted in drug development for cancer treatment (Yamamoto and Gaynor, 2001). Interesting background on the cancer cell killing properties of the stem barks, roots and leaves of *C. excavata* could provide avenues to investigate extensively on the basic mechanistic actions of cell death (Chinsembu and Hedimbi, 2006). Hence, further studies are required to investigate the anticancer activities of the extracts and chemical constituents isolated towards various cancer cell lines (Malek et al., 2011).

In one pharmacological study, Clausine-B was found to be active (IC₅₀<30 µg/ml) against four cancer cell lines tested (Zain, 2009). The IC₅₀ values for these four lines were determined to be 21.50 µg/ml (MDA-MB-231), 22.90 µg/ml (HeLa), 27.00 µg/ml (CAOV3) and 28.94 µg/ml (HepG2) respectively. Clausine-B was found to inhibit the MCF-7 cancer cell line at 52.90 µg/ml, but no IC₅₀ value was obtained against Chang liver (Brunner et al., 2009). A new carbazole alkaloid, 3-carbomethoxy-2-hydroxy-7methoxycarbazole, Clausine-TY, was isolated from the ethyl acetate extract of the stem bark of the Malaysian C. excavata. This new carbazole alkaloid showed significant cytotoxicity against CEM-SS cell line with IC₅₀ value of 44.80 µg/ml (Taufig-Yap et al., 2007). Four other natural pyranocoumarins which included clausenidin. nordentatin, clausarin, and xanthoxyletin were isolated from the Malaysian medicinal plant C. excavata (Chin et al., 2006). The first three compounds showed cytotoxic activity against four human cancer cell lines (A549, MCF7, KB, and KB-VIN) (Su et al., 2009). In a study conducted by Patil et al. (1995) to determine the major flavonoid compounds and the composition of essential oils predominant in C. excavata, and also to examine its antioxidant and anticancer activities, the flavonoids of crude extracts of the leaves and fruit of the plant were investigated. The study concluded that the antioxidant activity of flavonoids was associated with anticancer properties (Look et al., 1991). The MTT assay, however, showed fruit oil having better cytotoxic activity than that in methanol crude leaf extract against MCF-7 cells. This implied that C. excavata fruit oil might be a good source for breast cancer treatment due to the presence of possible active anticancer agents (Lewis and Elvin-Lewis, 1995). This result could partly explain the popularity of this plant in folk medicine as a remedy for cancer and HIV patients in the eastern part of Thailand as well as in Malaysia (Nakamura et al., 1998).

Antibacterial activity

It was reported that, four known coumarins, dentatin, nordentatin, clausenidin and xanthoxyletin, and six known carbazole derivatives, 3-formylcarbazole, mukonal, 3methoxycarbonylcarbazole, murrayanine, 2-hydroxy-3formyl-7-methoxycarbazole and clauszoline J were isolated from *C. excavata*. All compounds showed antimycobacterial activity at a minimum inhibitory concentration (MIC) of 50, 100, 200, 100, 200, 50, 100, 200, 50 and 100 µg/ml, respectively. O-Methylated clausenidin, prepared from 3, exhibited antimycobacterial activity at MIC 50 µg/ml (Sunthitikawinsakul et al., 2003)

Antifungal activity

Few studies have been conducted concerning the antifungal activity of phytochemicals of *C. excavata.* Three compounds from the aforementioned compounds in antibacterial activity section, namely 3-formylcarbazole, mukonal and 3-methoxycarbonylcarbazole showed antifungal activities with IC_{50} values of 13.6, 29.3, 9.5 and 2.8 µg/ml, respectively (Sunthitikawinsakul et al., 2003).

Anti-HIV-1 activity

limonoid, clausenolide-1-ethyl ether Α and two coumarins, dentatin and nor-dentatin, were isolated from C. excavata. Coumarins. dentain and nor-dentain. with their structures related to an anti-HIV-1 substance, (+)calanolide A, were obtained from the crude chloroform extract of the rhizomes (DiLella et al., 1986). Both induced toxicity to cells used in a syncytium assay for anti-HIV-1 activity (Hughes, 2003). In addition, three carbazole derivatives, O-methylmukonal, 3-formyl-2,7dimethoxycarbazole and clauszoline and J. pyranocoumarin, clausenidin, were isolated from the rhizomes and roots of C. excavata (Singh and Bodiwala, 2010). O-methylmukonal, isolated from this plant for the first time, has been reported currently to have anti-HIV-1 activity (Ravi et al., 2006). All compounds displayed anti-HIV-1 activity in the syncytial assay with EC(50) values of 12, 29.1, 34.2 and 5.3 µm, respectively, thus, exhibited potential therapeutic index (PTI) values of 56.7, 8.0, 1.6 and 7.0, respectively (Kongkathip et al., 2005).

Immunomodulatory activity

The term "immunomodulation" denotes a change or/and strengthening of the suppression of cellular indicators, and humoral immunity and nonspecific defense factors. The essence of immunomodulation is that a pharmacological agent acting under various doses and time regimens displaying an immunomodulating effect

(Manosroi et al., 2004). In vitro immunomodulatory activities of aqueous extract, acetone extract and the Thai folklore extract of C. excavata Burm. f. on mouse immune system were investigated. The phagocytic activity of macrophages and splenocyte proliferation in the absence and presence of mitogens (lipopolysaccharide, LPS) or pokeweed mitogen, PWM) were assayed. The results indicated that the aqueous extract exhibited maximum effect on both respiratory burst response and lysosomal enzyme activity more than the acetone extract and the Thai folklore extract; indicating effective phagocytic activation. For splenocyte proliferation assay, the Thai folklore extract with LPS gave maximum activity, higher than that with PWM, suggesting specificity towards B cell proliferation through T cell independent pathway similarly as LPS. Previous study had revealed the immunomodulating activity, which could explain the traditional use of this plant in Thailand as anti-malarial and anti-dysentery (Manosroi et al., 2003).

The effects of fractions from hot aqueous extract, acetone extract and the folklore preparation of C. excavata were studied on mouse splenocyte proliferation (Wang et al., 2008). The fractions of hot aqueous and acetone extracts were found to be the most active (Knio et al., 2008). On the contrary, the fractions from the crude folklore preparation resulted less active. This result could partly explain the popularity of this plant in folk medicine as a remedy for cancer and HIV patients in the eastern Thailand part of (Manosroi et al., 2004). Immunomodulating effects of the aqueous extract and the Thai folklore preparation (CEHF) of C. excavata Burm. f. in Balb/C mice (CEHW) were investigated.

Haemagglutinating antibody (HA) titers at day 0, 7, 14, 21, 28 and 35 from the serum of animals fed or injected intraperitoneally with the extracts for 5 days were compared and evaluated for humoral mediated immunity (HMI). Both extracts given orally reached maximum antibody titer at day 7, which was 2 weeks faster than by intraperitoneal administration (Manosroi et al., 2005). In a study conducted by Lin and Tang (2007) the effects of fractions isolated from hot aqueous extract, acetone extract and folklore preparations of *C. excavata* were studied on mouse splenocyte proliferation. The fractions of hot aqueous and acetone extracts were found to be the most active (Cazacu et al., 2003). On the contrary, the fractions from the crude folklore preparation resulted less active.

Antinociceptive activity

No antinociceptive activity has been reported except an investigation on an ethanolic extract of *C. excavata* leaves given orally at doses of 125.25 and 500 mg/kg body weight. The study showed significant antinociceptive activity on acetic acid induced writhing in

Common name	Chemical class	Plant Part	Bioactivity	Reference
Clausine-B	Carbazole alkaloid	Stem bark	Anticancer	(Wan, 2009)
Clausine-TY	Carbazole alkaloid	Stem bark	Anticancer	(Taufiq, 2007)
Clausenidin, nordentatin, clausarin, dentatin	Pyranocoumarins, coumarin	Root bark	Anticancer, antibactrialial	(Wu, 1994; Ali, 2000) (David PJ, 1991)
Dentatin	Coumarin	Roots		
Xanthoxyletin, murrayanine	Carbazole derivatives	Leaves	Antibactrialial	(Sunthitikawinsakul, 2003)
Mukonal	Limonoid	Stem bark	Antifungal	(Takemura, 2002)
Dentatin	Coumarin	Rhizomes	Anti-HIV-1	(Ali, 2000)
Clausene-D	Alkaloid	Leaves	Antiplatelet	(Wu, 1994)
Sansoakamine	Carbazole alkaloid	Stems	Anti-malarial	(Lastra, 2005)

Table 1. Summary of the most biologically active compounds of Clausena excavate.

mice (Rahman et al., 2002).

Antiplatelet activity

Clausine-D that was isolated from *C. excavata* exhibited an antiplatelet effect, which was mediated by inhibition of thromboxine A_2 formation. A higher concentration of Clausine-D (150 µm) was required to produce almost complete inhibition of collagen induced platelet aggregation (Wu et al., 1994).

Anti-malarial activity

Malaria continues to be a major infectious disease of the developing world and the problem is compounded not only by the emergence of drug resistant strains but also from a lack of a vaccine. Malaria is a disease caused by protozoans in the genus Plasmodium (Isaka et al., 2002). There are only 4 species of the Plasmodium that can infect humans; the remainder infects other mammals, as well as birds and reptiles. Among the 4 species, Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale and Plasmodium malariae infect humans. P. falciparum to a lesser extent and P. vivax account for the majority of 300 to 500 million clinical attacks per year, resulting in an annual death toll of 1 to 2 million. Most of these cases occur in African countries, especially among children younger than 5 years (Jaturapat et al., 2001). Few investigations have been done by the phytochemists concerning the anti-malarial activity of C. excavata (David and Wright, 1991). A new carbazole alkaloid, sansoakamine, which has been isolated from the stems of C. excavata showed moderate anti-malarial activity against P. falciparum with a MIC value of 6.74 µg/ml (Lastra-Gonzalez et al., 2005).

Insecticidal activity

Insecticidal activity is one of the interested biological

activities considered by researchers in the field of pharmacology (Knio et al., 2008). Few investigations also have been conducted based on the isolated pure compounds as well as crude extracts of C. excavata (Ravi et al., 2006). In one study, Fourth-instar larvae of Aedes aegypti and Aedes albopictus served as test organisms (Pitasawat et al., 2007). The larva colonies of the mosquito collected from the Kaoshiung strain were reared in the Department of Parasitology, Chang-Gung University, at 27 °C with a 12:12 h light : dark photoperiod in 80 ± 10% relative humidity. A 10% yeast suspension was used as food source (Cheng et al., 2009). In summary, Table 1 shows the most important biologically active compounds isolated from C. excavata and their pharmacological properties. In fact, most of the isolated chemical compounds from different parts of this plant are proved to be biologically active.

CONCLUSION

In broad terms, herbal drugs used in traditional medicine require further detailed investigation pertaining to ethnopharmacological approach. The present review explores the details of C. excavata, its botany, habitat, traditional uses, phytochemistry and pharmacology. Numerous studies have been conducted on different parts of this plant, but till today, the plant has not been developed or commercialized as suitable drugs by pharmaceutical industries. Therefore, a detailed and systematic study is required for identification, cataloguing and documentation of this plant, which may provide a meaningful way for promoting traditional knowledge of herbal medicinal plants. The present review revealed that the plant is used in treating various ailments. C. excavata contains different bioactive compounds from different chemical classes. Several parts of the plant have interesting anticancer, antimicrobial, antioxidant, anti-inflammatory, anti-malarial immunomodulatory and properties. Consequently, further studies on this plant should be

considered by researchers in phytochemistry and pharmacology in discovering newer and potential bioactive compounds as antioxidants and anticancers.

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