

PHCOG REV.: Plant Review

Chemical and Phytomedicinal Investigations in *Lunasia amara*

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ABSTRACT

Since the ancient period up to the current time, plants have been utilized by man as medicinal agents on the basis of ethnomedical and folkloric beliefs. Later on, these speculations were transformed into a scientific basis as single drug agents. Basic sciences working on plants serving as brewing pot for pharmaceutical leads, use obtained natural products as new bioactive pharmaceutical prototypes. The battle on the search for phytomedicines that are safe and affordable to the reach of the common population is still on-going. *Lunasia amara* Blanco is a small green shrub found in rain forests of the Philippines, Eastern Java, Borneo, Sulawesi, Moluccas, Papua New Guinea and Australia, and a member of the alkaloid-bearing tribe of the Family Rutaceae. It is a medicinal shrub well-known for its beneficial effects in treating stomach pains, snake bites, hypertension, diarrhea and for enhancing sexual aggressiveness. Quinoline alkaloids and sesquiterpenes have been identified to occur in this plant. Activities related to CNS, antimicrobial property and cytotoxicity have been associated with the quinoline alkaloids. This review aims to give an over-all picture regarding works that have been done on this medicinal plant as far as phytochemical and pharmacological aspects are concerned.

KEY WORDS : *Lunasia amara*, quinoline alkaloids, sesquiterpenes, Rutaceae, CNS activity, antitubercular

INTRODUCTION

Over one-third of the population in developing countries lack access to essential medicines. These countries use traditional medicine to help meet their primary health care needs. The World Health Organization launched its comprehensive traditional medicine strategy in 2002 to promote safe, effective and affordable traditional medicine. Safety and efficacy data are available for very few plants. In most countries, the herbal medicines market is poorly regulated, and herbal products are often neither registered nor controlled. The general public - consumer and health-care professionals alike - need updated, authoritative information on the safety and efficacy of medicinal plants (1). In the Philippines, a survey among herb doctors revealed that 7,500 plants were being used for various ailments. Wide acceptability of the use of plants for health care by low income groups in rural areas was shown in a survey conducted in Central Luzon (2). After many years of persevering efforts to develop a Pharmacopeia that would establish standards for drug products and crude plant drugs, the publication was realized in 2004, which included 51 monographs of crude plant drugs (3).

In response to the global need for scientific information on medicinal plants, the present review compiles all relevant information on the biological and chemical aspects of *Lunasia amara* Blanco, a plant used by Filipino herbalists for stomach troubles and snakebites (4-5). *Lunasia* species are small trees or shrubs, doecious, evergreen, and found in Eastern Java, Borneo, Philippines, Sulawesi, Moluccas, New Guinea, and Northern Queensland. There is one species, *Lunasia amara*

Blanco, quite variable, with a variety in Babuyan, a group of islands in northern Philippines (6). *Lunasia amara* Blanco has two varieties, var. *amara* and var. *babuyanica* (Merr.) Hartley. Synonyms of *Lunasia amara* Blanco var. *amara* are *Lunasia amara* var. *repanda* Merr., *Lunasia gigantifolia* Merr., *Pilocarpus amara* Blanco, *Rabelaisia philippinensis* Planch., *Lunasia philippinensis* (Planch.) F.-Vill., *Lunasia reticulata* Elm., *Mytilococcus quercifolius* Zoll., *Lunasia mollis* Merr., *Lunasia macrophylla* Merr., *Lunasia obtusifolia* Merr., *Lunasia nigropunctata* Merr., *Lunasia pubifolia* Merr., *Lunasia truncata* Elm. (6-8). *Lunasia amara* Blanco var. *babuyanica* (Merr.) Hartley has only one synonym, *Lunasia babuyanica* Merr. (6, 8).

Botanical description

The Rutaceae genus, *Lunasia* Blanco, is mostly Malesian of origin, spanning from the Philippines and south Borneo to Java and east through Guinea with the exception of its occurrence in the Cape York Peninsula of Australia. It can be clearly differentiated from other genera in the Rutaceae family as noted by its trimerous flowers that are displayed as small, head-like clusters. This genus is over-all variable in certain vegetative characteristics and species like *L. parvifolia*, *L. quercifolia*, *L. mollis* and *L. obtusifolia* are rightfully distinct from the neotype of *L. amara*. They thrive usually to lowlands and grows in habitats ranging from well-drained rain forests to re-grown gardens and dry thickets. Similar ecologic profiles and characteristics are exemplified in a number of well-noted Malesian plant species (9). *Lunasia amara* Blanco is an erect, sparsely branched shrub or a small tree to 12 m tall. Leaves are crowded toward

branchlet ends, on petioles 1.5-15 cm long, blades oblanceolate to obovate or elliptic or lanceolate, 5.5-60 cm long, chartaceous to coriaceous, pale green beneath, rounded to acuminate, at base cuneate to narrowly rounded or cordate; main veins 9-35 (or more) pairs; margins subentire to sinuate or sinuate-subdentate. Inflorescence to 25-28 cm long, the staminate wider than the pistillate. The twigs are smooth, but the young tips are olivaceous lepidote. Flowers in headlike clusters 3-6 mm diam., very small; sepals 0.5 mm long in staminate, to 1.5 mm long in pistillate flowers; petals greenish yellow to whitish, 1 mm long in staminate, 2-2.3 mm long in pistillate flowers. Fertile stamens 1 mm long. Fertile gynoecium 0.9 mm wide, not so high; styles 0.3 mm long;

stigma 0.5 mm long. Follicles obovate-truncate, somewhat flattened laterally, transversely ribbed on the sides, 6-15 mm x 5-10 mm, usu. beaked (5 mm). Seeds obovoid; testa dark or reddish brown, subglossy, papery (6). The fruit consists of three yellowish capsules, each of which is 1 centimeter or more in length, is plainly marked with ribs, and opens along the veins and upper sutures (Fig. 1 and 2). It is flowering and bearing fruits all year round. It is usually cultivated through plantation and seed propagation. It grows in soils ranging from ultramafic to limestone in well-drained rain forest, moist to rather dry thickets, gallery forest and secondary growth, from sea-level up to 900 m altitude (10).



A



B



C

(A) *Lunasia amara* shrub, (B) Leaves, (C) Branch with fruits (Photos courtesy of Ms. Xyza Templonuevo)



Figure 2 : . *Lunasia amara* (A)- A flowering branch, (B) – fruits, (C) Seed. (Ref. 5)

Lunasia amara var. *amara* ("lunas") is as described above, and is found on well-drained rainforests, moist to rather dry thickets, gallery forests and garden re-grown at low and medium altitudes, sea level to 900 m. (6). It is distributed throughout the Malesian region, except Babuyan, Philippines (6-8) (Fig. 3).

It is known in several Filipino vernacular names as apdongkahoi, lunas, lunas-bondok, saltiki, santiki, labau, pait (Tagalog); lubi-lubi (central Bisaya); labau (southern Luzon Bisaya); marmangga (Ibanag); dayangdang, paitan (Ilokano); pait, papait, bunglay (Bikol); lunan (Pampango); palatangan (Gadang) (7, 11).

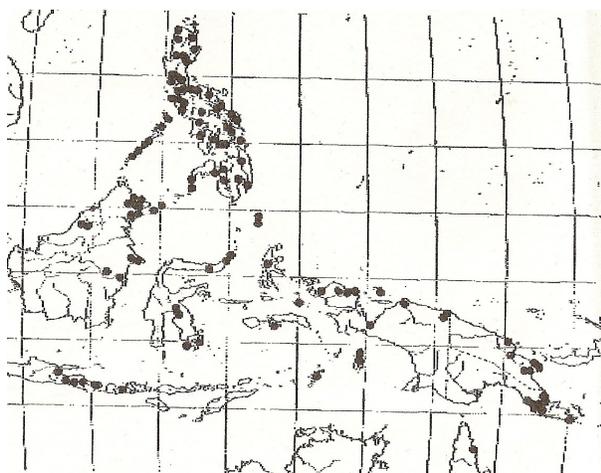


Figure 3 : Distribution of *Lunasia amara* in the Philippine islands, Java, East Borneo and Papua New Guinea. (Ref. 9)

Lunasia amara var. *babuyanica* (Merr.) Hartley ("Babuyan lunas" (Rojo 1999)) is a shrub to a treelet 2 m tall; branchlets, etc., with stellate hairs. Leaves with chartaceous, narrowly obovate blades 23-47 x 10-18 cm, apex obtuse to bluntly acuminate, base obtuse to rounded; main veins 21 or 22 pairs; margins repand toward apex. Follicles densely covered with twisted, simple or 2-3 branched stellate-pubescent processes to 8 mm long. It is endemic in Babuyanes (Camiguin Island, Cagayan Province, northern Philippines). It grows in thickets near seashores and in forested slopes of Camiguin Volcano at 400 m or lower near the seashore (6, 8).

Ethnopharmacology

Lunasia amara is a very popular and well-accepted ethnomedical herbal plant in the Philippines as well as in Indonesia. The early Philippine ethnomedical reports on *L. amara* are on the use of its bark and wood for stomach troubles and against snake bites (4, 10). The bark and seeds are used by herb doctors to cure gastralgia in general as well as certain adynamic conditions of the digestive organs (12). The leaves and the bark are used for stomach troubles (13-14). It is reported that upon ingestion of the herbal preparation of this plant, taken during digestive disorders, a very dilute aqueous solution in small amounts was known to effect or induce vomiting and cramps. The alkaloids have been observed to cause a fall of blood pressure in etherized cats (14). From the cortex has been extracted an alkaloid

with cardiac action. The leaves are employed in decoction in cases of gastralgia (15). The concoction of the bark is used for treating diarrhea, stomach pains and as antidote for snake bites in the Philippines (16). The bark is used to treat infected eyes (9). In Central Sulawesi, the sap is used for inflamed or irritated eyes (10).

Similar ethnobotanical reports indicate its use for gastritis and hypertension (17). In Papua New Guinea, the freshly prepared scrapings of the bark are applied to tropical ulcers (18). In Indonesia, a decoction of the bark and leaves is rubbed on swollen limbs, and is also used for treating skin diseases (10).

Lunasia amara is known as Sanrego in Indonesia. This stems from its first discovery by a farmer residing in Sanrego Village, Bone Regency, South Sulawesi Province (19). Ethnomedically, the plant is used by Indonesians to treat diabetes, food poisoning, malaria, skin diseases and snake bites. The crude drug is prepared by obtaining the hot water decoction of the stem barks (20).

Preparations and Commercialization

Lunasia amara Blanco is a significant component of the Indonesian herbal tonic known as Jamun Kuat Lelaki Lawang Sanga, in combination with Pasak bumi (*Eurycoma longifolia* Jack), *Panax ginseng* C. Meyer, *Tribulus terrestris* Linn. and *Zingiber aromaticum* Valetton. It is claimed that with exact and balanced constitution of this herbal preparation, it can increase sexual appetite, sexual passion, stamina, halts early ejaculation and extends period of sexual intercourse (21).

As a lone component in Sanrego, it is also marketed as Indonesian Herbal Viagra. This was confirmed by research studies done by Prof. Dr. Muchsin Darise, a senior pharmaceutical researcher at Hasanuddin University in Ujungpandang. Reports about Sanrego conducted since 1994 revealed that it is actually a good sexual stimulator (20).

EARLY PHYTOCHEMICAL STUDIES

Quinoline Alkaloids. A wide spectrum of molecular diversity in certain alkaloidal archetypes is observed in plant species belonging to the family Rutaceae. This rather curious chemical variety in a not so large plant family is associated with several unique biosynthetic pathways which identify several aromatic amino acids as molecular forerunners (22). It has been noted by Price (23) that there are in actuality ten types of alkaloid structures occurring in this family with anthranilic acid as the main precursor of most alkaloids identified so far. In addition to chemotaxonomic purposes, this group of natural products has been consistently investigated due to their wide array of biological effects.

The interest in the identification of the alkaloidal constituents of *Lunasia amara* roots from the pioneering works of Lewin (24) and Boorsma (25). In several phytochemical studies done on *L. amara*, four main groups of quinoline alkaloids were identified on the basis of their proposed biogenetic origins. This chemotaxonomic information also formed the basis of its botanical alignment in Rutaceae.

3-Dimethylallyl-2-Quinolones. This group of alkaloids is the most widespread and well-investigated. They are

biogenetically produced by the pre-condensation of anthranilic acid and acetate to afford quinolone, a highly oxidized quinoline derivative. Further biosynthetic transformation such as nucleophilic addition of 3,3-dimethylallyl (from mevalonic acid) gives 3-(3',3'-dimethylallyl)-4-O-alkyl-2-quinolone metabolites (22). In *L. amara*, six were identified belonging to this group. These are lunacridine and its hydroxy derivative (26), lunidine (27) and its hydroxyl derivative (26) and lunidonine (27). Another unidentified alkaloid falling along this class is lunolone (26). Hart and Price (28) described the possibility that lunidine and lunidonine are artefacts, in view of the observed positive optical rotation reported for lunidine. This idea was deduced from the easy transformation of (-)-lunine picrate to its dextrorotatory form in alkaline solutions (Fig. 4).

Furoquinolines. This type of nitrogenous metabolites is considered chemically simple. Most possess an aryl group and are usually variegated on the manner of ring substitution in the benzenoid structure and the presence of a methoxy group at C-4 (22). The two optically inactive quinoline alkaloids identified from *L. amara* of this type are kokusagine (7,8-methylenedioxydictamnine) and skimmianine (26). Both compounds were also detected in a species of *Orixa* (*Orixa japonica*), another alkaloid-bearing Rutaceae genus (29) (Fig. 5).

Furoquinolones. This group of basic compounds were classically known as artefactual natural constituents until they have been proven to occur naturally in several genera, on the basis of their basic ring structure either as simple furoquinoline or as 2-isopropyl-2,3-dihydrofuroquinoline. The genus *Lunasia* is considered as one of the major sources of the latter type together with the genera, *Ptelea* and *Balfourodendron*. This chemotaxonomic information places *Lunasia* to the Toddalioidae (sub-tribe Pteleinae) (22). The major isopropyl-bearing alkaloid metabolite isolated from *L. amara* is lunacrine along with minor amounts of lunine (26, 30). The latter compound has been described to occur also in *L. quercifolia*, an Australian *Lunasia* species (31).

Two additional minor alkaloids, having a hydroxyl group in their isopropyl moiety were also identified. These are

hydroxylunacrine and hydroxylunine (26). The former possesses a methoxy group on its benzenoid ring while the latter has a methylenedioxy moiety. In addition, an unidentified alkaloid, lunacrinol, was also isolated in a very small amount.

The fifth member of this group that was identified in *L. amara* is lunasine, a quaternary furoquinoline alkaloid (28). This water-soluble alkaloid was used as a reference in a biosynthetic hypothesis, whether 2-quinolones in *Lunasia* originate mainly from quinolinium ions during isolation and work-up, or they exist in the plant naturally (Fig. 6).

2-Arylquinolines and 4-quinolones. This group of alkaloids is derived biogenetically by condensation of anthranilic acid and other aromatic acids like phenylalanine and tyrosine (32). Along with the genera *Balfourodendron* and *Orixa*, the genus *Lunasia* is reported to contain such group of alkaloids. This information further gives evidence regarding the strong biochemical link between these three Rutaceae genera (22). The 2-arylquinolines identified from *L. amara* are 4-methoxy-2-phenylquinoline (33) and 4-methoxy-2-(3',4'-methylenedioxyphenyl)quinoline (graveolinine) (26) while the two 4-quinolones known so far from this plant are 2-phenyl-4-quinolone (also found in *L. quercifolia* (31) and *Casimiroa edulis*, a Mexican Rutaceae plant(34)) and lunamarine (26) (Fig. 7).

Sesquiterpenes. The sesquiterpenes are a large family of C-15 isoprenoid natural products found in higher and lower plants, microbes and some marine organisms. Many have biological activity, including antimicrobial, antitumour, and cytotoxic properties. In plants, they play important ecological roles in interactions with insects and microbes and act as attractants, deterrents, antifeedants and phytoalexins. Sesquiterpenes are key components of many essential oils, which are important commercially for the flavour and fragrance industries. When distilled from the plant matter, these compounds stimulate glands and the liver, and have anti-allergen, antispasmodic, and anti-inflammatory properties. The leaves of *L. amara* afforded an essential oil that is principally constituted of α -elemene, germacrene, bicyclogermacrene, bicycloelemene, β -bournene, α -elemene, α -farnesene and α -cadinene (35) (Fig. 8).

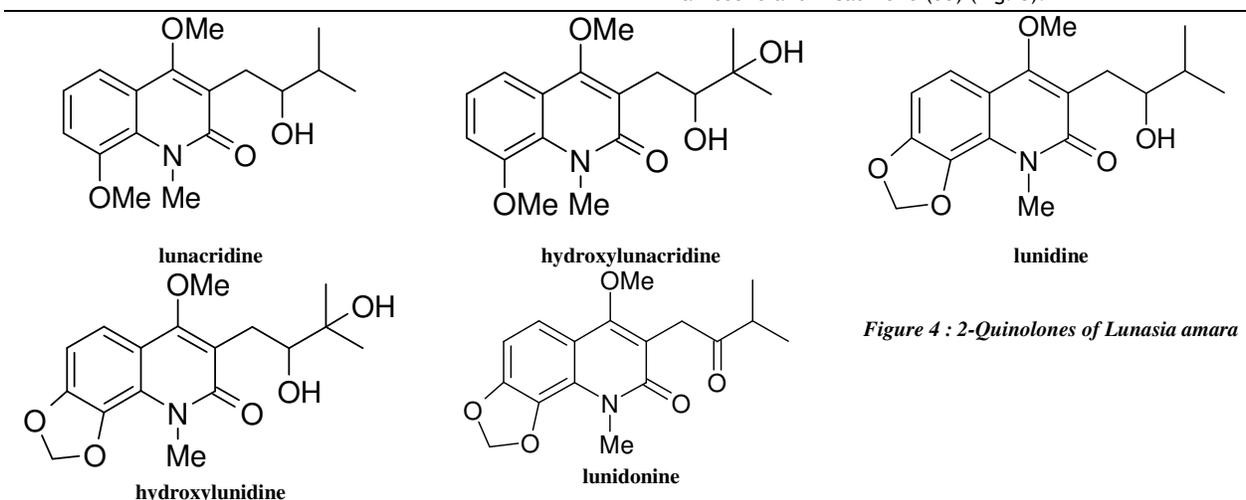


Figure 4 : 2-Quinolones of *Lunasia amara*

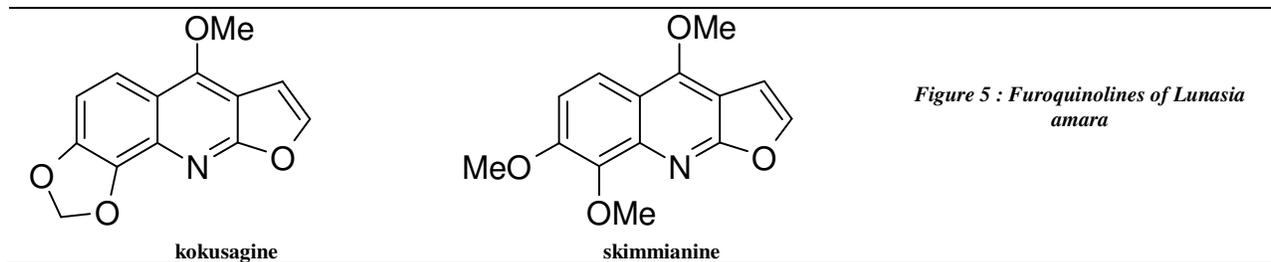


Figure 5 : Furoquinolines of *Lunasia amara*

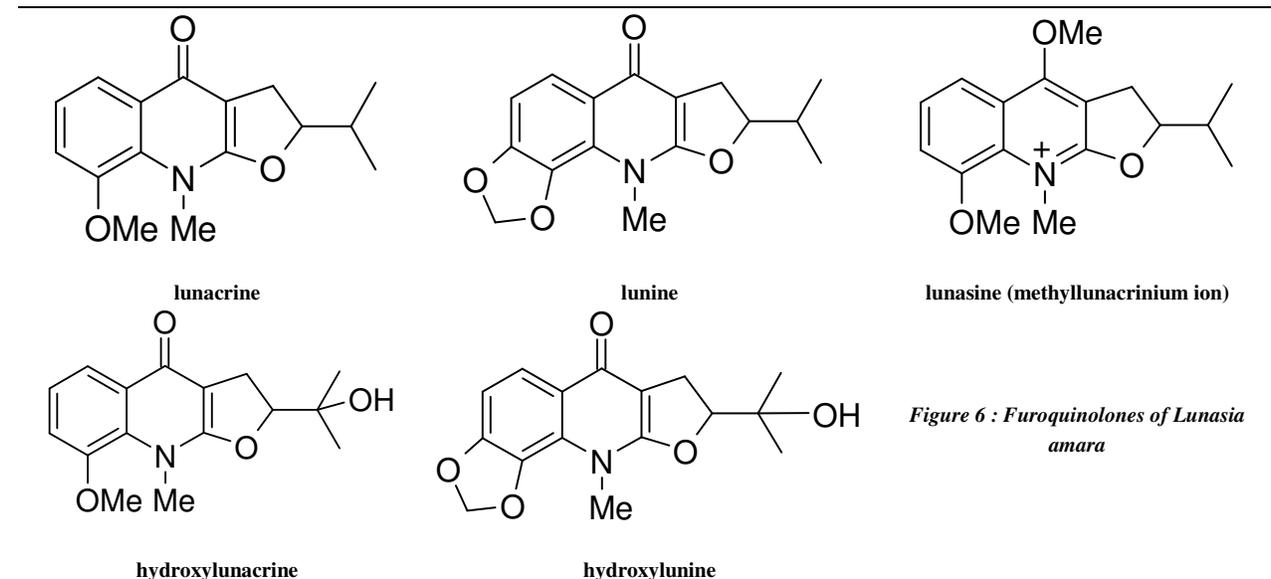


Figure 6 : Furoquinolones of *Lunasia amara*

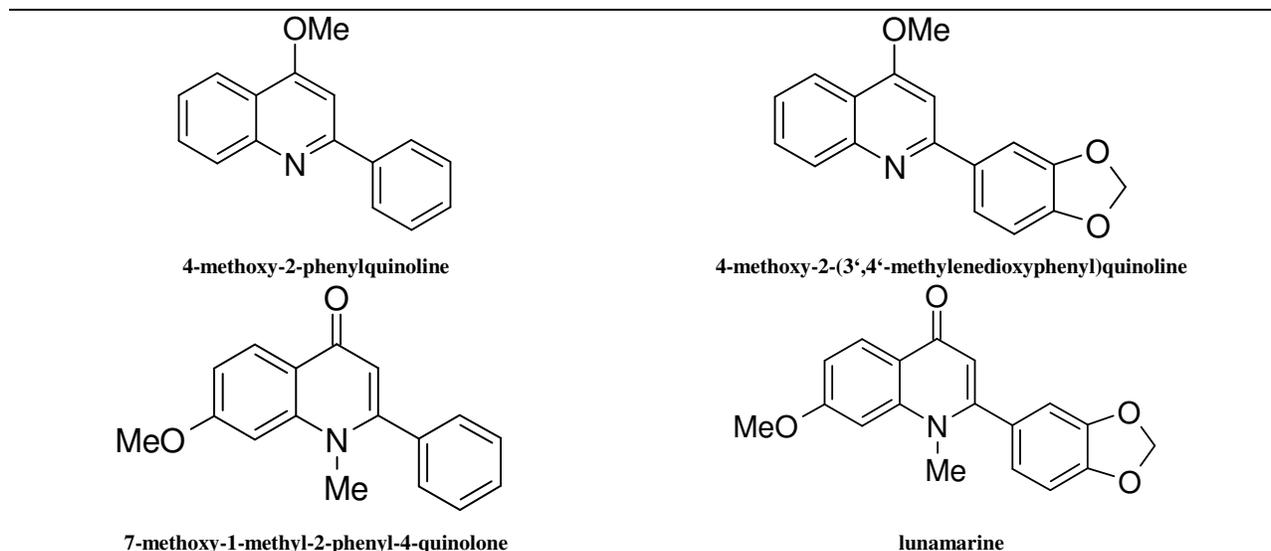


Figure 7 : 2-Arylquinolines and 4-quinolones of *Lunasia amara*

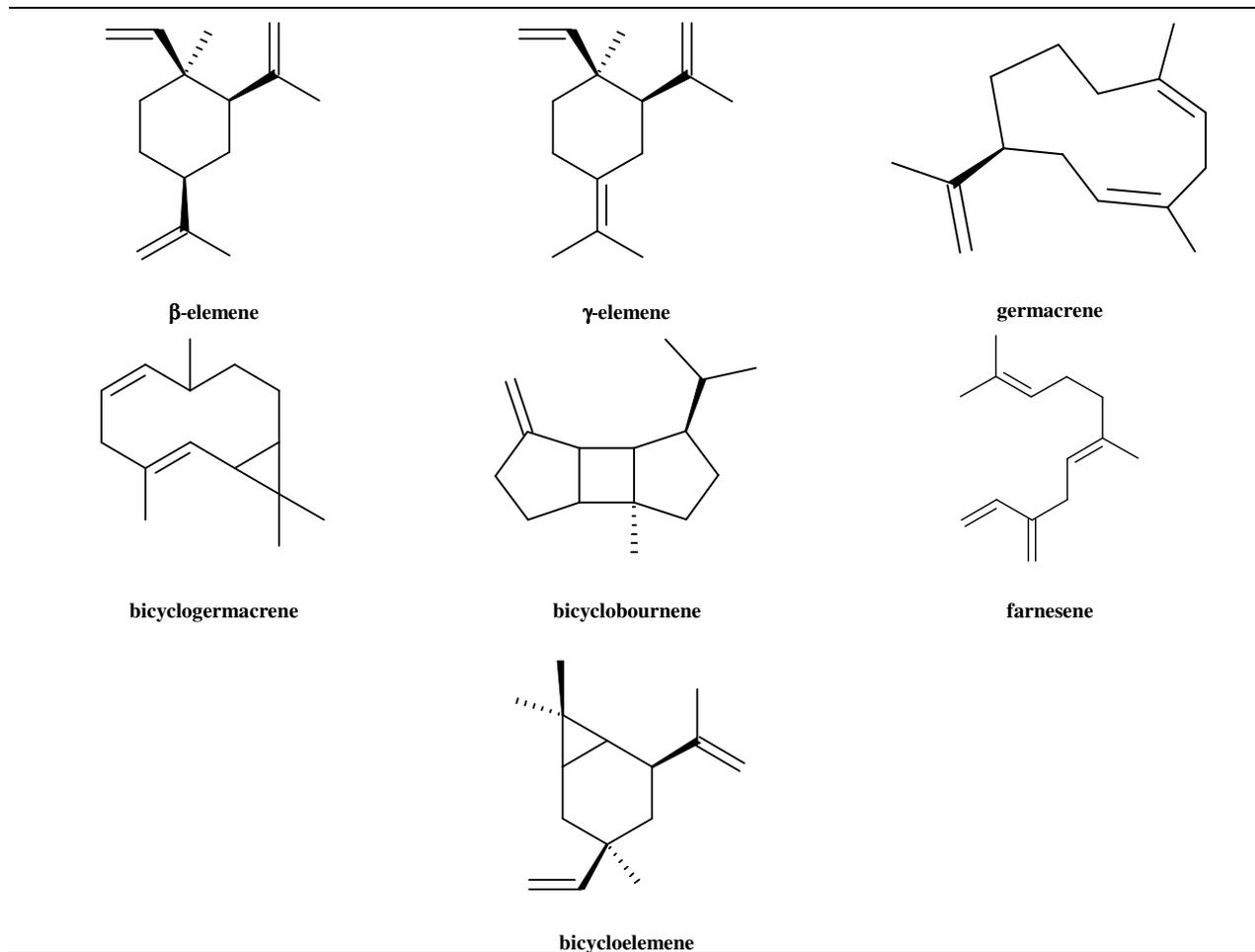


Figure 8: Sesquiterpenes in the essential oil of *Lunasia amara*

PHARMACOLOGICAL PROPERTIES

Few pharmacological studies have been reported for the extract and phytochemical constituents of *L. amara*. A summary of published biological activities is described below. **CNS activity.**

Lunacrine and lunasine show activity on muscles as proven by a continuous increase in tone and a rapid diminution of the power of response of the muscle to stimulation. This property on muscular tone is observed not only on isolated voluntary and smooth muscles but also on the blood vessel walls, where a significant contraction happens, and on the heart where a significant diminution in the contractions was noted. The toxic effect of these alkaloids is related to the stoppage of respiration concurrently with that of the circulation. Blood pressure experiments upon anaesthetized, decerebrated and decapitated animals display a sudden decrease which is mainly due to the action on the heart muscle. Lunasine, however, causes an augmentation in the decapitated preparations, exhibiting substantial action on the vasomotor centre which is not observed with lunacrine (36). This effect could be related to the presence of a methylenedioxy bridge in the alkaloids. This structural feature is also found in some known broad-spectrum P450 inhibitors, such as piperonyl

butoxide, piperine, myristicin and safrole. These findings can give leads for the discovery of new active modulators for the therapeutic effect of some drugs whose biotransformation pathway involves cytochrome P450 enzymes, e.g. barbiturates, several antibiotics and anticancer drugs (37). Lunamarine has been found also to decrease arterial blood pressure in cats and to have very low toxicity in mice (38).

Antibacterial activity.

Atkinson (39) and Collins (40) reported that an extract from a dried plant sample of Australian *L. amara* exhibited antibacterial properties when tested in a rapid direct plate method. To validate this result and determine the active components responsible for microbial inhibition and mode of action, Maciver and co-workers (18) went on investigating the bark for possible activity against *Staphylococcus aureus*. They suggested that an activity against this test organism could further verify the anti-ulcer ethnomedical claims for this plant since *S. aureus* together with other microbes like spirochaetes, fusiform bacteria and aerobic species are known to aggravate stomach infection.

It was found in their result that the active principle is lunacridine which was converted to its trifluoroacetyl form for stability reasons. This alkaloid is prone to cyclization

forming either a furan or dihydrofuran ring structure. No activity was observed with *Escherichia coli*. Further inhibition tests with penicillin, erythromycin and ciprofloxacin-resistants revealed activity against the latter strain. This was accounted to the close structural resemblance of lunacridine with quinolone antibiotics such as ciprofloxacin. This observation gave rise to the idea that this *Lunasia* alkaloid specifically acts on bacterial type II topoisomerases (18).

Antituberculosis activity.

In our quest to search for bioactive substances from Philippine plants that may possess activity towards the organism that causes tuberculosis, we embarked on the wide exploration of potential anti-TB plants that thrive in the rain forest of Subic Bay in Zambales, Philippines. We found that *L. amara* is one of the plants that display a promising activity against *Mycobacterium smegmatis* ATCC 607, as observed in a preliminary screening assay (41). Setting for more in-depth phytochemical studies and collaborative validation of its antimycobacterial property (BACTEC radiorespirometric assay), we were able to purify and identify the bioactive constituents and these are graveolinine, 4-methoxy-2-phenylquinoline and kokusagine. The two 2-aryquinoline alkaloids were observed to have a lower minimum inhibitory concentration compared to the furoquinoline alkaloid, kokusagine. A comparative analysis revealed that an aryl group, such as a phenyl or a methylenedioxyphenyl ring at the C-2 position of the quinoline nucleus marks an enhanced inhibitory activity (42). Also, a 4-methoxy group and a fully aromatized quinoline nucleus effects good activity (43-44).

DNA intercalation.

Intercalation happens when ligands of suitable size and chemical nature install themselves in between base pairs of DNA. These ligands are mostly polycyclic, aromatic, and planar, and are often good nucleic acid stains. Lunacridine possesses characteristics of a good ligand capable of intercalating with DNA due to its planar positively charged structure in acidic media. Lunacridine and its trifluoroacetyl derivative were observed to display DNA intercalating potential as seen in their ability to displace ethidium bromide, a known cationic ligand intercalator. The latter compound was found to be more active due to stability reasons (18).

Topoisomerase II decatenation.

A major role of DNA topoisomerase II *in vivo* is to catalyze the cleavage of double-stranded DNA, allowing passage of a second DNA duplex through the break. This activity requires adenosine triphosphate (ATP) and is necessary for separating catenated DNA duplexes found at the end of replication. The decatenation of DNA molecules is a topoisomerase II-specific reaction, and is a convenient assay for measuring topoisomerase II activity *in vitro*. Maciver et al. (18) reported that the trifluoroacetyl derivative of lunacridine exhibits decatenating activity.

Cytotoxicity.

With the observed ability of 2'-O-trifluoroacetyl lunacridine to inhibit human topoisomerase II at a very low concentration, it was postulated by Maciver and co-workers

(18) that this compound is also a potential anticancer agent (45). And true it was, the compound displayed cytotoxic activity against a non-carcinoma lung cell fibroblast, ATCC MRC-5, and two carcinoma cell lines, namely, NCI H226 and HeLa. The activity was ascribed to properties observed for topoisomerase toxins such as anthracyclines and ellipticines.

Caspase activation.

Caspase activation plays a central role in the execution of apoptosis. A concentration dependent activity against caspase 3/7 was observed for 2'-O-trifluoroacetyl lunacridine (18). These caspases carry an important duty in apoptotic cell death as effector caspases which act when cell damage occurs as a result of topoisomerase II induced poisoning, giving broken double strands of DNA.

SUMMARY AND CONCLUSION

Hundreds of plants worldwide are used in the traditional treatment of various ailments and diseases. Some of these have undergone *in vitro* screening but the efficacy of such herbal preparations has seldom been rigorously proven in controlled clinical trials. Conventional drugs provide effective therapeutic property for certain panels of diseases but for antibiotics, there is an increasing issue of drug resistance, and consequently, a further need to discover new bioactive natural products. Although natural products are not necessarily safer than the synthetic analogues, still many patients undergoing treatment choose herbal medicines. Hence, healthcare professionals should be aware of the available pharmacologic evidence of several herbal preparations. In this review, we have presented information on the botanical description, ethnopharmacology, pharmacology, and phytochemistry of *Lunasia amara*, a medicinal shrub found in Southeast Asia and Cape York Peninsula of Australia. A variety of quinoline alkaloids and several sesquiterpenes have been reported for *Lunasia amara*. As for its pharmacologic aspects, hypotensive or CNS-related activity, antibacterial (including antituberculosis or antimycobacterial), mechanistic cytotoxic property and erectile alleviation have been described for this Rutaceae medicinal plant. With the limited plant chemistry and biological activities of the alkaloids in *L. amara*, there are still more areas to explore. As elaborated in several published papers, quinoline alkaloids of several genera related to *L. amara* display an array of biological effects like suppression of aggressiveness, sedation (46), prevention of heart arrhythmia, suppression of atropine-induced psychosis (47), antidiuresis, antimicrobial (46) and antitumorogenic activity (48). They are also known to inhibit LDL oxidation (antioxidant) (49), exhibit antiviral (against hepatitis B virus and HIV) (50-51)), antifungal (52), antiplatelet aggregation (53), antileishmanial (54-55), antiprotozoal (56), molluscicidal (57), estrogenic effect (58), antiparasitic (59), analgesic, antipyretic (60), and antimalarial activity (61). Skimmianine, a component of *L. amara*, was found to produce DNA lesions (62), kill Hepa B virus and possess estrogenic effect (63). To end, further research is needed to fully realize the potential of *L. amara* as a phytomedicinal agent and more scientific effort especially along clinical investigations (64) has to be invested

for its efficacy as an herbal antibiotic and as a preparation with Viagra-like property.

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