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Research Article

Anti-Cancerous Potentiality of Coumarins of *Mesua assamica* (King & Prain) Kosterm. – An Endemic Plant of Assam

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ABSTRACT

Plants are part of our daily diet, and the phytochemicals and their nutritional value have been intensively studied for decades. Besides producing essential primary metabolites (e.g. carbohydrates, lipids and amino acids), higher plants also synthesize a wide variety of secondary metabolites which has therapeutic value. The present review aims to aggregate the scientific research data available on anti-cancerous activity of coumarins isolated from *Mesua assamica* plant which is endemic to Assam in India. It is a slow growing tall evergreen tree belonging to family Clusiaceace. The bark of the plant has been valued in traditional medicine for anti-malarial activity and treating fevers. It has been examined for the cytotoxic activity based on a panel of human cancer cell lines, and for the anti-malarial activity against chloroquine sensitive and resistant clones of *Plasmodium falciparum.* Coumarins and coumarin-derivatives have been isolated and characterised from root barks and fruit peels, which serves as potent anti-cancer agents. There needs more extensive study to understand the biosynthesis pathway of the bio-molecules so that bio-technological tools can be used for exploitation and drug development. Besides that, the plant needs attention for conservation of the habitat which is limited to the unique physico-geographical conditions of Assam.

Keywords: *Mesua assamica,* Coumarins, Anti-cancerous, Cytotoxic, Anti-malarial.

INTRODUCTION

Coumarins

The isolation of coumarin is dated back to 1820, first reported by Vogel in Munich. The chemical was first isolated from *Coumarouna odorata* Aube (*Dipteryx odorata*), and thus named coumarin. By 1868 it was known to have the molecular formula $C_9H_6O_2$ with molecular weight of 146.15 Da. Coumarins are lactones with the basic structure of 1, 2-benzopyrone[1]. The phenylpropanoid biosynthetic pathway is known to synthesize a variety of compounds along with those involved in plant defence against pathogenic organisms like coumarins. The key steps in this pathway mostly include P_{450} enzymes and about more than 15, P_{450} dependent reactions have been characterized in this pathway[2]. Coumarins comprise a very large class of derivatives of phenols found in plants and they are consisted of fused benzene and α-pyrone rings. Substitution can occur at any of the six available sites and the possible permutations offered by substitution and conjugation is the reason for so many coumarin derivatives present in nature[3]. Coumarin is a naturally occurring secondary metabolite in several plant families and essential oils which is used as fragrance in food and cosmetic industry. The importance of coumarins is not only for their medicinal properties but their occurrence in medicinal plants can also be used to distinguish between closely related species or to detect adulterations of plant preparations[4].

Plant Description

Mesua assamica (King & Prain) Kosterm. belonging to family Calophyllaceae is commonly known as *'Sia Nahor'* in Assam and the tree is found along the foothills of the Himalayas in the North Lakhimpur subdivision of Assam, India[5]. The local name of the species in Myanmar is thera-phi. It is regarded as highly prized medicinal plant, and was described by Burmese bard Nawadaygias as heavenly precious flower in one of his poems[6].

Taxonomic classification

Kingdom -Plantae
Division -Tracheophyta
Class -Magnoliopsida
Order -Malpighiales
Family -Calophyllaceae
Genus -*Mesua*
Species -*M. assamica* (King & Prain) Kosterm.

Basionym

Kayea assamica King & Prain

Synonyms

Kayea acuminatissima Merrill
Kayea assamica Prain
Kayea acuminatissima (Merrill) Kosterm.

Common name

Assamese -Sia-Nahor
Thai -Theraphi

Habitat and Distribution

It is a slow growing tall handsome evergreen tree much resembling Nahor (*Mesua ferrea*) in general habit[1].

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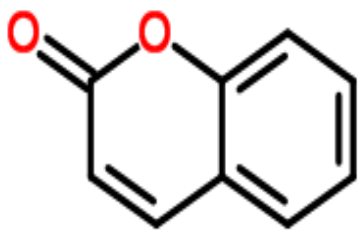

Figure 1: Chemical Structure of Coumarin.

Its distribution is found in India (Assam), Myanmar [Burma] (Kachin), peninsular Malaysia (Kedah, Kelantan, Trengganu, Pahan, Negeri Sembilan, Johor) and Borneo[7].
The plant has been reported from Dullung and Kakoi Forest Reserves of Lakhimpur district of Assam in India[8].

Morphology

Bark is light brownish grey in colour.

Leaf is 3-5.5 by 1-1.8 inches in dimension, ovate or elliptic-lanceolate in shape, shortly acuminate and often very finely mucronate leaf tip, cuneate at the base, firmly coriaceous texture and somewhat shining above and dull underneath.

Flower is white, about 0.3 inches across, terminally arranged in axillary fascicled panicles 3-6 inches long with short, decussate, slender glabrous, bracteate branches; pedicels in flower very slender, 0.1-0.2 inches long, much enlarged and thickened in fruit; bracteolate, bracts and bracteoles are small, opposite and caducous; buds are globose about 0.08 inches in diameter. Sepals 4, in 2 pairs, with imbricate arrangement, the outer about 0.15 inches in diameter, orbicular shaped, inner one of spathulate shape, both of which becomes much enlarged in fruit so as to completely envelope it. Petals 4 in number, white in colour, about 0.1 inches long, sub-orbicular shaped and thin. Stamens are numerous in number, longer than the sepals; filaments free and capillary; anthers are small, globose shaped. Ovary 1-celled; style is slender; stigma 4-fid; ovules 4 and erect.

Fruit is depressed and globose shaped, scarcely 1 inches long but about 1.8 inches in diameter, entirely or almost entirely enveloped by the accrescent hard sepals.

Seed is solitary, globose shaped but very depressed with 1-1.3 inches in diameter; testa reddish brown, crustaceous, smooth, and cotyledon is fleshy[1].

RET status

It has been described as endemic plant by Botanical Survey of India. But looking into the restricted distribution within the state of Assam itself the plant should be categorised as endangered plant species.

Ethnobotanical uses

M. assamica fruits are used as a fish poison and the aqueous extract of the stem bark is valued for its anti-malarial effects. The pollen is used for sores, fistulas, fever, and malaria. It has been reported to be used to reduce extreme hotness in the body, dizziness, dry skin, and fever[9,10].

Pharmacological Characterization

Recently, the coumarins reported from *M. assamica* of Myanmar has been identified for its potential anti-cancerous properties. Bioassay-guided fraction and isolation from the flower of *M. assamica* of Myanmar led to the isolation of nine novel coumarins, together with nine known ones. Among them five novel coumarins has been reported, 100% preferential cytotoxicity(PC_{100}) against human pancreatic cancer cell line, PANC-1cells under nutrient-deprived medium[10].

Isolation of Coumarin from Air Dried Bark

Four coumarin derivatives theraphins A, B, C, D along with three known xanthones, 2-hydroxy-xanthone, 1, 7-dihydroxy-xanthone and 5-hydroxy-1-methoxy-xanthone were isolated from the EtOAc-soluble extract of the bark of *M. assamica.*

Theraphins A, B, and C, have exhibited strong cytotoxic activity against human colon cancer (Col2), epidermoid carcinoma of the nasopharynx(KB), and hormone dependent human prostate cancer(LNCaP) cell lines with IC_{50} values in the range of 3.5-13.1 µM. Cytotoxic activity against Lu1 cell line varied significantly, with IC_{50} values in the range of 7.5-42.8 µM. Theraphin D, a pyranocoumarin, exhibited very weak activity with an IC_{50} value of 52.2 µM against only the KB cell line.

It was suggested from the preliminary biological assessment that the 7-hydroxyl group must play important

Table 1: Biological Activity of Isolated Compounds from different parts of *M. assamica*.

Plant Material	Isolated Compounds	Biological Activity
Air Dried Bark	Theraphin A-D	Theraphin A, B and C shows strong cytotoxic activity Theraphin D has weak cytotoxic activity[6]
Air Dried Root Bark	Assamene	Antifungal Properties [9]
Air Dried Fruit Peels	Mammea A/AA cyclo F (Cyclomammeisin) Mammea A/AA (Mammeisin)	Cyclomammeisin shows cytotoxic activity. Mammeisin inhibits oxidative phosphorylation [9]
Flower	Kayeassamins A-I Mammea A/AA cyclo D Mammea A/BC Mammea B/AC Mammea A/AC Mammea A/AC cyclo D Theraphins B and C Mammea B/AC cyclo F Deacetylmammea E/ BA cyclo D	Kayeassamins A, B, D, E and G displayed100% preferential cytotoxicity[10]

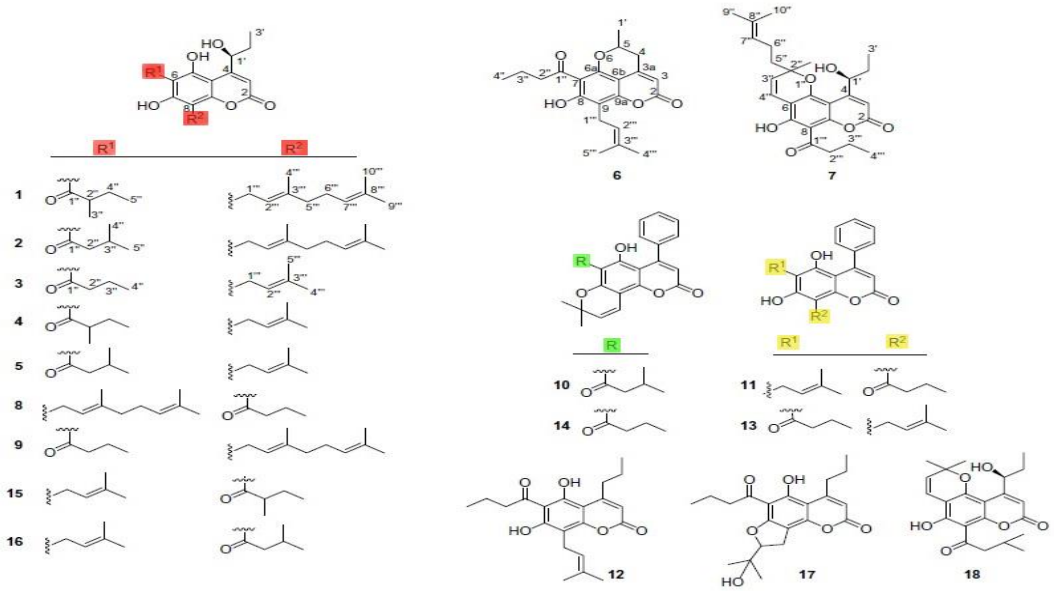

Figure 2: Structure of isolated Coumarins (1-18) from *M. assamica* flower [10].

Table 2: Cytotoxic Activity* of Theraphins[6].

Compound	Cell Lines			
	Lu1	Col2	KB	LNCaP
Theraphin A	7.5	7.2	3.5	3.5
Theraphin B	16.2	7.2	5.7	3.4
Theraphin C	42.8	13.1	6.2	6.4
Theraphin D	>53.8	>53.8	52.2	>53.8

*Data are given as estimated IC_{50} values in µM.

role for cytotoxic activity of coumarin derivatives on the basis of the report that 7-hydroxycoumarin, which is a major human metabolite of coumarin, shows growth inhibitory effect on human malignant cell lines in vitro.

The coumarin derivatives were also evaluated for antimalarial activity against Chloroquine-sensitive (D6) and Chloroquine-resistant (W2) clones of *Plasmodium falciparum*. There was a modest activity shown by the coumarin derivatives with IC_{50} values in the range 9.7-11.1 µM against the D6 clone, and IC_{50} values in the range 5.1-10.4 µM against the W2 clone[6].

Isolation of Coumarin from Air Dried Root Bark and Fruit Peels

The concentrated petrol extract of air dried root bark of *M. assamica* afforded a compound which was designated by the name Assamene, which on acetylation form an acetate derivative whose spectral data were very similar to an insecticidal coumarin isolated from *Mamea americana*. The examination of the air dried fruit peels of *M. assamica* afforded two known coumarins, Mammea A/AA cyclo F (cyclomammeisin)and Mammea A/AA (mammeisin).

Assamene, cyclomammeisin and mammeisin have shown appreciable antifungal properties against pathogenic fungi *Helminthosporium oryzae*, *Phytophthera oryzae*, *Alternaria solani*, *Curvularia eraglostidis* and *Colletotrichum gleosporioides.* Mammeisin shows inhibition of oxidative phosphorylation at 0.5µg/mL. Cyclomammeisin shows cytotoxic activity against cell line KB at ED_{50} 6 µg/mL[9,11].

Isolation of Coumarin from Flower

Bioassay-guided fraction and isolation of the $CHCl_3$-soluble fraction of 70% EtOH extract of the flower of *M. assamica* of Myanmar led to the isolation of novel coumarins, kayeassamins A–I, together with nine known ones [mammea A/AA cyclo D (10), mammea A/BC (11), mammea B/AC (12), mammea A/AC (13), mammea A/AC cyclo D (14), a mixture of theraphins B and C (15 and 16), mammea B/AC cyclo F (17), and deacetylmammea E/ BA cyclo D (18)].

Five novel coumarins, kayeassamins A (8), B (9), D (2), E (3), and G (5), displayed PC_{100} against PANC-1 cells under nutrient-deprived medium at 1µM. The order of potency for the other isolates were 4, 12 (2µM) > 1 (4µM) > 11 (8µM)> 13 (16µM) > 10, 18 (32µM) > 6, 7, 15–17 (64µM) > 14 (> 256µM).

Upon studying the structure and activity relation, it was observed, compounds possessing an isoprenyl or a geranyl substituent at C-8 and a hydroxypropyl substituent at C-4 showed the most potent activity. Interchange of isoprenyl and any acyl groups between C-6 and C8 significantly lowers activity even in the presence of a hydroxypropyl group at C-4. Replacement of the hydroxypropyl group at C-4 by a phenyl group or presence of any additional cyclic ring in the coumarin nucleus also leads a dramatic loss of activity[10,12,13].

CONCLUSION

M. assamica is a plant with potent bioactive molecules of pharmacological importance. There is need to understand the biosynthesis pathway of the bio-molecules so that bio-technological tools can be used for exploitation and drug development. Isolation of bio-active molecules from wild or cultivated plants is more economically feasible than chemical synthesis of the same. Biotechnological production in plant cell cultures is an attractive alternative, but there has been only limited commercial success

Table 3: Antimalarial Activity* of Theraphins[6].

Compound	*P. falciparum* clones		Cytotoxicity	SI**	
	D6	W2	KB	D6	W2
Theraphin A	9.7	7.7	3.5	0.36	0.45
Theraphin B	9.8	9.6	5.7	0.58	0.59
Theraphin C	9.5	5.1	6.2	0.65	1.22
Theraphin D	11.1	10.4	52.2	4.70	5.02
Chloroquine***	0.012	0.13	54.5	4542	149

*Data are given as estimated IC_{50} values in µM.
**SI = KB IC_{50} /*P. falciparum* IC_{50}
***Positive Control

because of lack of understanding how these metabolites are synthesized. State of the art genomics tools can be used to enhance the production of known target metabolites or to synthesize entire range of novel compounds by so-called combinatorial biochemistry in cultivated plant cells. The plant also needs better conservation strategy in the light of its limited vegetation in unique physico-geographical conditions of Assam. Bioassay guided fraction and isolation of the coumarin molecules and their anti-carcinogenic activity has necessitated the need for extensive research along with conservation of the plant by the scientific community and the society for the future.

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CONFLICT OF INTEREST
The author declares no conflict of interest.

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