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 Clusiaceae. 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 C9H6O2 with molecular weight of 146.15 Da. Coumarins are lactones with the basic structure of 1, 2-benzopyrone1. 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 P450 enzymes and about more than 15, P450 dependent reactions have been characterized in this pathway2. Coumarins comprise a very large class of derivatives of phenols found in plants and they are consisted of fused benzene and α-pyrene 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 nature3. 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 preparations4.

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, India5. The local name of the species in Myanmar is thera6. It is regarded as highly prized medicinal plant, and was described by Burmese bard Nawadaygias as heavenly precious flower in one of his poems6. 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 habit1.

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

**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 and thickened in fruit so as to completely envelope it. Petals 4 in number, white in colour, about 0.1 inches long, sub-ornicular 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 sepal. Seed is solitary, globose shaped but very depressed with 1-1.3 inches in diameter; testa reddish brown, crustaceous, smooth, and cotyledon is fleshy.

**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.

**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 against human pancreatic cancer cell line, PANC-1 cells under nutrient-deprived medium.

**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>
<thead>
<tr>
<th>Plant Material</th>
<th>Isolated Compounds</th>
<th>Biological Activity</th>
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<tbody>
<tr>
<td>Air Dried Bark</td>
<td>Theraphin A-D</td>
<td>Theraphin A, B and C shows strong cytotoxic activity</td>
</tr>
<tr>
<td>Air Dried Root Bark</td>
<td>Assamene</td>
<td>Theraphin D has weak cytotoxic activity</td>
</tr>
<tr>
<td>Air Dried Fruit Peels</td>
<td>Mammee A/AA cyclo F (Cyclomammeisin)</td>
<td>Cyclomammeisin shows cytotoxic activity.</td>
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<tr>
<td>Flower</td>
<td>Kayeassamins A-I</td>
<td>Mammeisin inhibits oxidative phosphorylation</td>
</tr>
<tr>
<td></td>
<td>Mammee A/AA cyclo D</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mammee A/BC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mammee A/AC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mammee A/AC cyclo D</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Theraphins B and C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mammee B/AC cyclo F</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Deacetylmammea E/ BA cyclo D</td>
<td></td>
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</tbody>
</table>

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

**Figure 1: Chemical Structure of Coumarin.**

**Figure 2: Distribution Map of *M. assamica***

**Figure 3: Chemical Structure of Mammeisin.**

**Figure 4: Chemical Structure of Theraphin D.**
Table 2: Cytotoxic Activity* of Theraphins.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cell Lines</th>
<th>Lu1</th>
<th>Col2</th>
<th>KB</th>
<th>LNCaP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theraphin A</td>
<td>7.5</td>
<td>7.2</td>
<td>3.5</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Theraphin B</td>
<td>16.2</td>
<td>7.2</td>
<td>5.7</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>Theraphin C</td>
<td>42.8</td>
<td>13.1</td>
<td>6.2</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>Theraphin D</td>
<td>&gt;53.8</td>
<td>&gt;53.8</td>
<td>52.2</td>
<td>&gt;53.8</td>
<td></td>
</tr>
</tbody>
</table>

*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.

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, Mameea A/AA cyclo D (10), mammee A/BC (11), mammee B/AC (12), mammee A/AC (13), mammee A/AC cyclo D (14), a mixture of theraphins B and C (15 and 16), mammee B/AC cyclo F (17), and deacetylmameea 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.

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 biotechnological 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 Theraphins6

<table>
<thead>
<tr>
<th>Compound</th>
<th>P. falciparum clones</th>
<th>Cytotoxicity</th>
<th>SI**</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>D6</td>
<td>W2</td>
<td>KB</td>
</tr>
<tr>
<td>Theraphin A</td>
<td>9.7</td>
<td>7.7</td>
<td>3.5</td>
</tr>
<tr>
<td>Theraphin B</td>
<td>9.8</td>
<td>9.6</td>
<td>5.7</td>
</tr>
<tr>
<td>Theraphin C</td>
<td>9.5</td>
<td>5.1</td>
<td>6.2</td>
</tr>
<tr>
<td>Theraphin D</td>
<td>11.1</td>
<td>10.4</td>
<td>52.2</td>
</tr>
<tr>
<td>Chloroquine***</td>
<td>0.012</td>
<td>0.13</td>
<td>54.5</td>
</tr>
</tbody>
</table>

*Data are given as estimated IC50 values in µM.
**SI = KB IC50 / P. falciparum IC50.
***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.

REFERENCES