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Medicinal Application of Naphthyl-Iso-Quinoline Alkaloid from Ancistrocladus – A Review

KEYWORDS: Naphthyl iso-quinoline alkaloids, Ancistrocladus, Anti-HIV, Anti-malarial, Anti-tumor, Anti-microbial,

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ABSTRACT *Ancistrocladus is a tropical liana plant. All species of Ancistrocladus are known to have different types of Naphthyl iso-quinoline (NIQ) alkaloids. NIQ alkaloids are unique because their basic structure comprises of a biaryl system consisting of naphthalene and a tetra hydro quinoline moiety and these alkaloids display atropisomerism due to the bulky ortho-substutients adjacent to the biaryl axis. NIQs highly unusual structure is due to an unprecedented biogenetic origin, for which a polyketide pathway has been assumed. Researchers have shown that these alkaloids exhibit interesting biological activities, such as Anti-HIV, Anti-malarial, Anti-tumor, Anti-microbial and spasmolytic activity. This review reports various initial attempts to study the medicinal property of NIQ alkaloids found in genus Ancistrocladus.*

1.0 Introduction:

Ancistrocladus is genus of lianas restricted to Africa and S.E. Asia, belongs to family Anaistrocladaceae and is found in low land to premontane and wet to seasonal forests. Ancistrocladus is notable for its climbing habit assisted by hooks on the stem and its nuts like fruits (Fig. 1)

Figure – 1: *Ancistrocladus heyneanus* (A) Leaves (B) Hooks and (C) Fruits

Importance of this plant came into light after the Anti HIV potential was discovered in *Ancistrocladus korupensis (*African species). The species was named *A. korupensis* by Thomas and Gereau of the Missouri Botanical Garden after Korup Park, located in a 60 million years old tropical rainforest (Cragg *et al* 1996). In 1990, researchers at the National Cancer Institute (NCI) discovered that extracts from *A. korupensis* inhibit the ability of HIV to kill human cells. Later Mc Mahon *et al*, (1995) described the mode of action of the anti HIV compound. In 1998, because of the toxicity found in Michellamine B, NCI stopped conducting R&D on *A. korupensis* (Thomas et al, 1993). Govindachari et al (1971 – 1997) initiated research on *Ancistrocladus heyneanus* (the only known Indian species) and he predicted the potential applications of Naphthyl iso-quinoline (NIQ) alkaloids present in this plant.

2.0 Unique Alkaloids from Ancistrocladus

Presence of NIQ-alkaloids in all the species of Ancistrocladus have been reported by Govindachari's group, which comprise of two moieties; a Naphthyl group and an isoquinoline group. NIQ-alkaloids are unique due to their unusual substitution pattern, including an unprecedented methyl substitution pattern at C-3, a meta-oxygenation pattern at C-6 and C-8 a stereo chemically interesting biaryl linkage- connecting the iso-quinoline part to the Naphthyl moiety. This unusual structure arises from acetic acid units and not from aromatic amino acids like most of the alkaloids. Because of such unusual structures, remarkable biological activities have been shown by NIQ-alkaloids of Ancistrocladus e.g. anti-malarial (Hallock et al 1997), anti-HIV (Bringmann et al 1998) and anti-tumor activity (Bringmann et al 2008). Hallock et al (1997) isolated and examined two new monomeric NIQ-alkaloids Yaoundamine A and B from *Ancistrocladus Korupensis*, and found it to be active *in vitro* against the malarial parasite *Plasmodium falciparum*. The structures of these two alkaloids were determined by extensive chemical and spectroscopic analyses. The absolute configuration at C-3 was established by chemical degradation, while the axial chirality was deduced by comparison of experimental and calculated CD spectra. Yaoundamine B contains an unprecedented L rhamnose carbohydrate domain while Yaoundamine A is devoid of such moiety. Ancistrocladus has also shown presence of many NIQs, some of them (Fig. 2) that has medicinal properties are: Michellamine, Yaoundamine, Ancistrocladine, Ancistroheynine, Ancistrogriffithine and Ancistrogriffine.

3.0 Medicinal Applications of NIQ-Alkaloids

80% drug molecules are natural products or natural compound inspired (Harvey 2008). Traditional medicines (Ayurvedic, Unani, Tibbetan or Tribal) are from plants. Medicinal plants contribute to the modern treatment of cancer by either providing the active substance or template for synthesis or synthetic modification resulting in more effective anti-cancer agents (Matsumoto, 2008).

First evidence of *Ancistrocladus korupensis* leaves, collected from Malaysia, having, an active anti-AIDS agent Michellamine B was presented by Manfredi et al. (1991) and

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Boyd et al. (1994).

3.1 Anti-HIV activity of Michellamine

HIV is very prevalent and alarmingly increasing in Africa. Many natural products such as alkaloids, phenolics and terpenes have been found to have anti HIV activity. Michellamine (Fig. 2) in *Ancistrocladus korupensis* has exhibited anti HIV property. The Michellamine (Molecular Formula C46H48N2O8) vary from the structures of previously known NIQ-alkaloid in three important ways

1) Having a unique linkage site joining naphthalene to the iso-quinoline ring;
2) They are the only members to be found as oxidatively coupled dimmers;
3) Contains highest degree of non-methylated free phenolic oxygen of any of this family of alkaloids.

Michellamine can be either a homodimer or hetero-dimer. There are various isomers of Michellamine, namely Michellamine A, B and C. Michellamine is a representative of new class of biologically important quarter aryls. Structurally, it is characterized by the presence of no less than 6 free phenolic hydroxy groups and 2 secondary amino functions and stereo-chemically by the existence of 4 stereo center and 3 axes, one of which is configuratively unstable, whereas the other 2 are stereogenic due to the restricted rotation. Still, the molecule seems relatively simple, it is a C2-symetric dimmer consisting of 2 constitutionally and stereo-chemically halves and both moieties of such monomeric NIQ-alkaloids are likely to be derived biogenetically from identical polyketide precursors, as obvious from biomimetic cyclization reaction of B-polycarbonyl compounds. Acquired Immuno deficiency Syndrome (AIDS) is the life threatening stage of infection with human immuno deficiency virus (HIV).

Average MichellamineB content in leaves is 2.1l% dry wt. The highest levels of Michellamine-B were found in mature leaves, younger and older-fallen brown leaves have significantly less NIQ-alkaloid (Simon et al. 1995). Hollick et al (1997) found another Michellamine D - F, to be new HIV-Inhibitory dimeric NIQ-Alkaloids. No other alkaloid from Ancistrocladus has shown anti-HIV activity so far.

3.2 Anti-Tumor/Cancer and cytotoxic activity of NIQ-alkaloids

Cancer is a diseases in which abnormal cells divide without control, are able to invade other tissues and can spread to other parts of the body through the blood and lymph systems. There are more than 100 different types of cancer. Because of high death rate associated with cancer and the serious side effects of chemotherapy and radiation therapy, more and more research is being done on alternatives like plant extracts that show anti-cancer activity. Many medicinal plants possess immuno-modulatory and antioxidant properties, leading to anti-cancer activities. The immuno-modulatory activity is by stimulating both non-specific and specific immunity.

Prior to testing anti-cancer activity of any molecule it is imperative that its cytotoxicity should be assayed, hence, this section also deals with the cytotoxicity of NIQ-alkaloids.

Cytotoxicity assesses whether the compound is harmful for normal cell line or not. The development of *in vitro* cytotoxicity assays has been driven by the need to rapidly evaluate the potential toxicity of compounds, to limit animal experimentation and to carry out tests with small quantities of compound. There are 3 basic parameters upon which these assays are based:

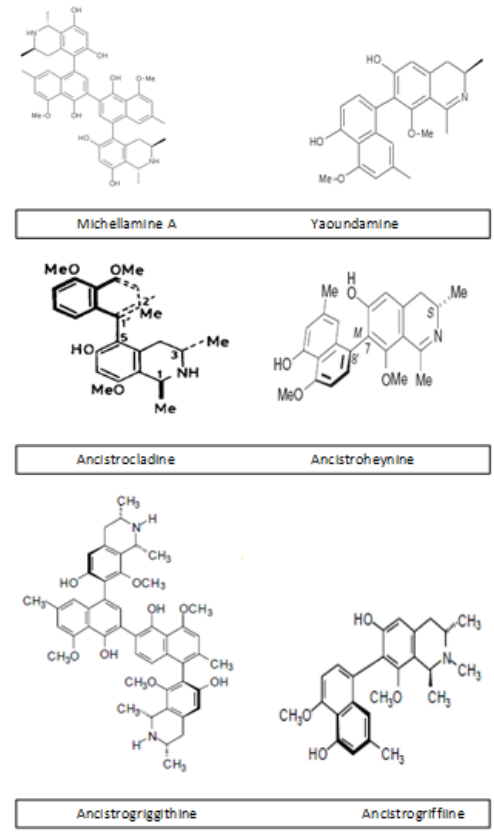

Figure – 2: Various Naphthyl iso-quinoline alkaloids that have shown different medicinal properties

Measurement of reduction in cellular metabolic activity as an early indication of cellular damage by measuring cellular ATP levels or mitochondrial activity via MTS metabolism.

Measurement of cell membrane integrity. The cell membrane forms a functional barrier around the cell, and traffic into and out of the cell is highly regulated by transporters, receptors and secretion pathways. When cells are damaged, they become 'leaky' and this forms the basis for the second type of assay. It is determined by measuring lactate dehydrogenase in the extracellular medium. This enzyme is normally present in the cytosol and cannot be measured extracellulary unless cell damage has occurred.

Direct measurement of cell number, since dead cells normally detach from a culture plate and are washed away in the medium. Cell number can be measured by direct cell counting, or by assassing total cell protein or DNA, which are proportional to the number of cells.

Karn et al (2014) have demonstrated non-cytotoxicity *and* anticancer activity of Ancistrocline extracted from the stem of *Ancistrocladus heyneanus* using MTT Cell Proliferation and Viability Assay. Here metabolic events lead to apoptosis or necrosis, a reduction in cell viability. The tetrazolium compound MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) reacts with mitochondrial enzyme Succinate dehdrogenase. The rate of tetrazolium reduction is proportional to the rate of cell proliferation. The NIQ-

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alkaloid were found to be non-toxic to Vero cells, as the %viability was found to be higher than HeLa cells at all concentrations of NIQ-alkaloid. With increase in concentration of NIQ-alkaloid %viability of HeLa cells decreases as compared to Vero cells . The IC50 value of Vero cells and HeLa cells were approximately $1x10^5$ ppm and $8x10^4$ (Fig. 3) ppm respectively.

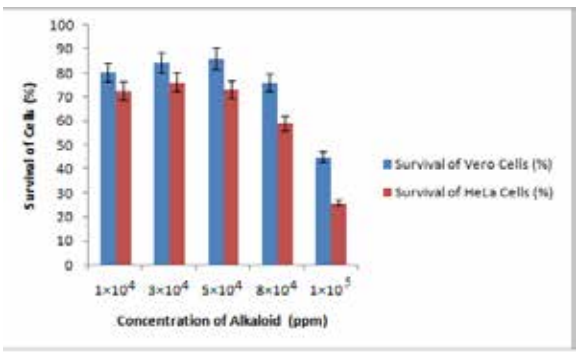

Figure 3: Histogram depicting impact of NIQ-alkaloid from stem of A. heyneanus on growth of Vero and HeLa cell lines *(Courtsey A.K. Karn, R. Dharmatti, M. Sharon* and *Madhuri Sharon*, 2015,

NIQ-alkaloid Ancistrocline from *A. tectorius* has also exhibited anticancer activity (Bringmannn & Kinzinger1992). Later Bringmannn et al (1997) found Betulinic acid (a naturally occurring pentacyclic triterpenoid) isolated from *A. heyneanus* having anti-retroviral, anti-malarial and anti-inflammatory properties. Recently Chowdhury (2002) found that it has potential as an anti-cancer activity too, by inhibiting topoisomerase. Bringmann et al (2008) have isolated 7 natural naphthoquinones from callus culture of *A. abbreviates*. One of these alkaloids Ancistroquinones B, C, D, E and F strongly induced apoptosis in human tumor cells derived from B cell lymphoma and multiple myeloma, without any significant toxicity towards normal peripheral mononuclear blood cells.

3.3 Anti-Malarial activity of NIQ-Alkaloids

Malaria is an infectious disease caused by a female mosquito bite, which introduces *Plasmodium* through saliva into the blood. Through the blood circulatory system, *Plasmodium* travels to the liver where it matures and reproduces. Malaria in severe cases can progress to coma or death. Five species of *Plasmodium* are known to infect humans i.e. *P. falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi* .

Currently no effective vaccine exists for malaria. However, a variety of anti-malarial medications are available e.g. quinine, artemisinin derivative artesunate mefloquine. Resistance has developed to several anti-malarial drugs. Hence, there is a demand for new anti-malarial drugs because malaria parasites, especially *Plasmodium falciparum*, have become resistant to chloroquine and other used drugs. Until now, only artemisinin and its derivatives appear to be near to the stage of commercial availability.

Since Ancistrocladus is used in the traditional medicine of West Africa for the treatment of fevers, malaria, it appeared to be worthwhile to evaluate their in vitro anti-plasmodial activity. NIQ-alkaloids from, *A abbreviates* and *A. berteri* were evaluated positive against *P. falciparum in vitro* by Francoise et al (1994). The NIQ-alkaloids were tested against the asexual erythrocytic stages of two strains of *Plasmodium falciparum* in vitro (k1/chloroquine-resistant and NF 54/64, clone A1A9/chloroquine-sensitive). Incorporation of 3H-hypoxanthine was measured in the presence of the test substances after 42 h of incubation at 37°C the alkaloids displayed activity. Moreover, Bringmannn et al (1996) showed that Ancistroheynine-A displays pronounced *in vitro* activity against *Plasmodium falciparum*. In 1997 Hallock et al isolated and examined two new monomeric NIQ-alkaloids Yaoundamine A and B from *Ancistrocladus Korupensis*, and found it to be active *in vitro* against *Plasmodium falciparum*. Simultaneously Francoise et al (1997) also demonstrated NIQ- alkaloid's anti-parasitic activity in *Plasmodium berghei* infected mice and Bringmann et al (1997) isolated lupane-type triterpene betulinic acid for the first time from *Ancistrocladus heyneanus*, which exhibited moderate to good *in vitro* antimalarial activity against asexual erythrocytic stages of *Plasmodium falciparum*. Moreover, Ancistrocladine was found weakly anti-plasmodial. To enhance the anti-plasmodial activity Bringmannn et al (2002) synthesized a new a constitutionally unsymmetric bis-naphthylisoquinoline, the unnatural `dimer' of the NIQ-alkaloid ancistrocladine, jozimine B; did exhibit enhanced antimalarial activity.

Karn et al (2014) extracted Yaoundamine from stem of *Ancistrocladus heyneanus*, and tested against two strains of *Plasmodium falciparum a Chloroquinesensitive strain 3D7* and the other *Chloroquine-resistant strain K1*. Based on the IC50 value and SI (Selective Index), it was found to exhibit promising anti-malarial activity against both 3D7 and K1 strains. They concluded that the extract is active against Chloroquine resistant parasites and efficacy is comparable to drug Arteether.

3.4 Anti-Microbial activity of NIQ-Alkaloids

Higher plants have capacity to produce a large number of secondary metabolites (organic chemicals) of high structural diversity. Secondary metabolite especially alkaloids are proved to be responsible for the antimicrobial activity of plants. Though they may not be essential part of plant metabolism itself, however, they play an important role in plant's defense system and give protection against pathogens and herbivores (Harborne 1984]). According to Aiyegoro-Okoh (2009) Antimicrobial compounds from plants, when used concurrently with standard drugs they enhance the drug activity.

Based on their mechanism of function such metabolites could be chemotherapeutic, bacteriostatic, bactericidal or antimicrobial. This fact is the directive force behind all the herbal therapeutics. Over the last 20 years, a large number of plant species have been evaluated for their antimicrobial activity. Root, stem and leaves of one of the plants known for having medicinal uses in African traditional system of medicine is *Ancistrocladus korupensis.* Current problem with synthetic antibiotics is development of multiple antibiotic resistances by disease-causing microorganisms. More et al (2012) isolated four different NIQ alkaloids from leaf and Karn et al (2014) from stem of *Ancistrocladus heyneanus* and tested their antimicrobial activity against gram negative and gram positive bacteria and showed considerable antimicrobial activity against both of them. Recently Aswathanarayan and Rai (2013) have also shown antimicrobial activity in the crude extracts of *A. heyneanus.*

4.0 Conclusion

Isolation of a natural active compound is the first stage in the development of a new drug. This study demonstrates the possibility of using Ancistrocladus as source of isolating various NIQ-alkaloids for therapeutic purpose such as

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Michellamine for treating HIV, Yaoundamine for malaria, ancistrocline for cancer, and various other NIQ-alkaloids as antimicrobial compounds and justifies the applicability of Ancistrocladus as used in traditional medicine in Africa and India

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