



2. RESEARCH ENVISAGED

Review of literature in the preceding section reveals the contribution of natural products of plant origin in the development of anticancer chemotherapeutic/ chemopreventive agents. Medicinal plants once served as the only source of therapeutic agents and today also, they continue to play a dominant role in the primary health care of the world's population. Although, it is generally believed that most of the modern drugs are of synthetic origin, yet many important ones are still extracted from plants. In last few years, there is renewed interest in evaluation of medicinal plants for identification of their chemopreventive/ chemotherapeutic potential against cancer. The plants rich in polyphenols, flavonoids and other antioxidants are of prime importance in development of chemopreventive agents against cancer (*Vide supra*).

In the Indian system of medicine there are a number of plants used for various oxidative stress induced diseases. One such plant is *Acacia catechu* Willd. (Fabaceae (alt. Leguminosae) subfamily: Mimosoideae) which is used extensively in folk and traditional system of medicine in India and Asia. It is said that the name 'catechu' was given to it because its bristles resemble the claws of animals of the cat family or may be because its heartwood contains cutch. The Sanskrit word 'Khadira' literally means that which alleviates the diseases and stabilizes the body. Khadira has various synonyms in ancient scriptures of Ayurveda, like balapatra tini leaved; vakrakanta has hooked spines, dantadhavana useful for cleansing the teeth, kanthi beneficial for the throat, kusthaghna anti dermatosis, etc. The great sage Charaka has categorized it as udarka prasamana antiurticarial and kusthaghna antidermatosis. Acharya Vagbhata has highly praised it as the drug of choice for the treatment of numerous skin diseases. Susruta has described the plant to be effective as an anti obesity herb [Sharma, 1985].

Most of the people in Kerala use boiled Khadira water (karingali vellam) for drinking purpose. There are number of Ayurvedic formulations in which *Acacia catechu* is used as an active constituent, e.g., 'Khadiradi Vati' used to treat throat infection and dental stomatitis, 'Khardirarishta' for the treatment of tumors, obesity, heart and skin diseases, 'Khadiradi

Gutika' for otitis, throat and tonsillar infections, 'Madhumehari' for diabetes and nephritis, and 'Dashmularishta' nourishes & rejuvenates the tissues, maintains healthy female reproductive system and remove toxins from the blood [Sharma, 1985]. There are a number of ayurvedic taila (oil) formulations which contain Khadira as one of the active ingredients [Dubey *et al.*, 2009]. *Acacia catechu* is highly valuable for its powerful astringent and antioxidant activities. It is commonly known as Katha which is an indispensable ingredient of Pan that is betal leaf preparation chewed in India. It is useful in dental, oral, throat infections and as an astringent for reducing oozing from chronic ulcers and wounds. Heartwood of khair is boiled with other ingredients to prepare the decoction. It is perceived as a tea for pregnant women to maintain their body heat. It is also given to treat fever due to cold during pregnancy. The water boiled with the heartwood chips of khair, is used to take bath by women after delivery. It is considered useful for the treatment of body pains. The concentrated aqueous extract known as khair gum or cutch is an astringent, cooling and digestive, beneficial in cough and diarrhea. Catechu is included in mouthwashes and gargles for gingivitis, stomatitis, pharyngitis, and oral ulcers [Singh and Lal, 2006]. It is also useful in cases of mercurial salivation, bleeding or ulcerated or spongy gums, hypertrophy of the tonsils, relaxation of the uvula, aphthous ulceration of the month. Applied externally to ulcer, boils and skin eruptions and is used extensively in Ayurvedic formulations [Kirtikar and Basu, 1993]. It is used in the treatment of passive diarrhea either alone or in combination with cinnamon or opium [Chaudhri *et al.*, 2012].

The bark of *Acacia catechu* in combination with other drugs is prescribed for snake bite [Chaudhri *et al.*, 2012]. The seeds of the plant are reported to possess hypoglycemic activity in rats [Singh *et al.*, 1976]. *Acacia catechu* also shows hypotensive effect [Sham *et al.*, 1984]. The decoction of bark mixed with milk is taken to cure cold and cough. *Acacia catechu* has been commonly used in India as an ointment for indolent ulcers and has been used in rural Bangladesh as a component of an anti-fertility pill. Other uses include arresting nose bleeds, assisting healing in nipple fissures, and acting as a contraceptive. A mixture of flower tops,

cumic, milk and sugar is useful in gonorrhoea [Lakshmi and Rajendran, 2012]. An infusion of the flowers and leaves is taken for gastrointestinal inflammations. The flowers are also sedating. Decoctions made from the powdered leaves, stems, and pods are taken for shigella, malaria, dysentery, and diarrhoea. The roots make a mucilaginous tea which possesses both antibacterial and anti-inflammatory. It helps soothe mucous membranes from the mouth through to the anus, reducing inflammation and attacking microbial infections [<http://www.ecoplanet.in/Herbsandplants/Acaciacatechu.htm>].

Catechonic acid or Khersal, a crystalline form of cutch sometimes found deposited in cavities of the wood is used as a remedy for chest diseases, especially for the treatment of asthma, coughs and sore throat. The bark is said to be effective against dysentery, diarrhoea and in healing of wounds. The seeds have been reported to have an antibacterial action [Kirtikar and Basu, 1993].

Acacia catechu is used in skin disorders, itching problems, diseases of mouth, teeth and throat, cough, obesity, worms, diabetes, fever, vitiligo, wound, bleeding disorders, anemia, eruptive boils, filaria and is used as rasayana (longevity enhancer). In Chinese medicine it is used for poorly healing ulcers, weeping skin diseases, oral ulcers, with bleeding and traumatic injuries [Chauhan *et al.*, 2011]. A small piece of cutch can be dissolved in the mouth to stop bleeding gums or heal canker sores. A mixture of catechu and myrrh (Kathol) is usually prescribed as a tonic and as a galactagogue to women after confinement [<http://www.cloverleaffarmherbs.com/acacia/#sthash.MZXDGkFs.oKRedelj.dpbs>].

Flavonoids constitute the most important single group of dietary phenolics and include catechins, proanthocyanins, anthocyanidins, flavones, and flavonols and their glycosides. Flavonoids are known to possess cancer chemopreventive properties. *Acacia catechu* heartwood contains significant amount of polyphenolic flavonoids which exhibit powerful antioxidant activities. It is evident from the facts mentioned above that *Acacia catechu* possesses many medicinal properties. However, it is surprising that the plant was never tested for its anticancer properties. One of the suggested mechanisms involved in the onset of cancer is

oxidative stress caused by an imbalance in free radicals, leading to DNA damage. Under these conditions, the resulting DNA damage may trigger the process by which a normal cell becomes a cancerous one. In addition, a number of bacteria and viruses have been implicated in the etiology of cancer and free radicals are involved in both the initiation and progression of cancer, it seemed worthwhile to scrutinize the action of *Acacia catechu* and its active constituents which is known to have antimicrobial and antioxidant properties.

The main objectives of this study are:

- *In-vitro* cytotoxicity testing of different extracts of *Acacia catechu* heartwood against MCF-7, A431 and HepG2 cancer cell lines.
- *In-vivo* anticancer effect of aqueous extract of *Acacia catechu* against multiorgan carcinoma.
- Phytochemical analysis of aqueous extract of *Acacia catechu* heartwood.
- Evaluation of anticancer effect of (+)-catechin against multiorgan carcinoma, *in-vitro* and *in-vivo*, and elucidation of possible anticancer mechanism.

Cytotoxicity assays are used widely in cancer research to screen natural and synthetic compounds for their anticancer properties. Cytotoxicity can be monitored using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay. This assay measures the reducing potential of the cell using a colorimetric reaction. Cytotoxicity can also be measured by sulforhodamine B (SRB) assay which determine total protein mass. Assessing cell membrane integrity by lactate dehydrogenase assay is one of the most common ways to measure cell viability and cytotoxic effects. The nature of each assay is different which adds credence to the accuracy of the data. Thus, these assays were employed in this study to evaluate the anticancer potential of *Acacia catechu* heartwood.

Following *in-vitro* cytotoxicity testing, the most effective extract of *Acacia catechu* heartwood i.e. aqueous extract (AQCE) was tested for its anticancer potential *in-vivo* using animal models for breast, skin and liver cancer. The *in-vivo* anticancer potential was evaluated through studying various parameters including tumor markers, oxidative stress and histopathology.

Once first two objectives were achieved, the most effective extract of *Acacia catechu* heartwood was phytochemically evaluated by using High Performance Liquid Chromatography (HPLC). The most active compound i.e (+)-catechin was isolated and evaluated for its anticancer potential against multiorgan carcinoma.

Treating cells with the anticancer compound can result in a variety of cell fates. The cell may undergo necrosis, in which they lose membrane integrity and die rapidly as a result of cell lysis. The cell can stop actively growing and dividing, or the cells can activate apoptosis. The mechanism of action of many anticancer drugs is based on their ability to induce apoptosis. Agents suppressing the proliferation of malignant cells by enhancing apoptosis may constitute a useful mechanistic approach to both cancer chemoprevention and chemotherapy. Under this objective, the mechanism of chemotherapeutic drug mediated cell killing of (+)-catechin will be evaluated by studying different apoptotic pathways. To do so, mRNA and protein expression levels of gene groups that regulate apoptosis was studied *in-vitro* and *in-vivo*. Further, the alteration in the induction of proteins that regulates apoptosis was studied by immunohistochemistry (IHC). Thus, the mechanism of cell death of (+)-catechin was evaluated.