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MEDICINAL PLANTS FOR COLORECTAL CANCER

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ABSTRACT
Colorectal cancer (CRC) is one of the most common, best-understood neoplasms from a genetic point of view. Each Year more than 1 million new cases of colorectal cancer are diagnosed worldwide. CRC is the 3rd most common malignancy and 4th most common cause of cancer mortality worldwide. CRC is also the 2nd most common cause of cancer deaths in the United States and other developed countries. Plants have been used for therapeutic purposes for centuries. Herbal plants and medicine is based on the fact that plants contain natural substances that can promote health and alleviate illness. Human beings rely on traditional medicine for their primary health care needs and most of this therapy involves the use of plant extracts or their active components. Various plants that have hypolipidemic, antiplatelet, antitumor or immune- stimulating properties that can be useful adjuncts in helping reduce the risk of cancer. Some wide variety of plants active phytoconstituents, including the flavonoids, terpenoids, lignans, sulfides, polyphenolics, carotenoids, coumarins, saponins, plant sterols, curcumins, and phthalides have been identified.

KEYWORDS: Colorectal Cancer, Malignancy, phytoconstituents.

INTRODUCTION
Cancer is a genetic disorder involving dynamic changes in the genome leading to uncontrolled cell growth, cell division, ability to invade to distant organs through lymph or blood and metastasize. There are more than 100 different types of cancer. Most cancers are named for the organ or type of cell in which they start - for example, cancer that begins in the in renal proximal cells is called renal cell carcinoma, cancer that begins in colon is called colon cancer; cancer that begins in basal cells of the skin is called basal cell carcinoma.[1]

In essence, human cancer is a genetic disease. The first implications of a genetic basis for cancer are endorsed to David Hansemann and Theodor Boveri. They both observed abnormal numbers of chromosomes arising by multipolar mitoses and suggested that this abnormality is the cause of tumor formation.[2]

Cancer is predicted to be progressively an important cause of morbidity and mortality all over the world. If the global cancer rates remain unchanged, the estimated incidence of 12.7 million new cancer cases in 2008 will rise to 21.4 million by 2030.[3]

The origin of the word is credited to the Greek physician Hippocrates who is considered as the "father of Medicine". Hippocrates used the terms carcinos and carcinoma to describe non-ulcer forming and ulcer forming tumors respectively. The word originated from the Latin for crab, because the swollen veins around a surface tumor appear like the legs of a crab. The exposure of chemical has been associated with the development of cancer as observed by John Hill in 1761, that snuff users developed nasal cancer more frequently than the general population [4]. Observed that occurrence of scrotal cancer in English chimney sweeps is due to the exposure of chemical present in soot. It was later declared as Polycyclic Aromatic Hydrocarbons (PAHs). More importantly, he also proposed a mechanism to reduce the incidence of these cancers simply by requiring these individuals to bathe on a regular basis.[5] Astute clinical observations have been the basis for the discovery of many of the currently known classes of chemical carcinogens in humans. Examples include the observation workers in the aniline dye industry in Germany frequently developed bladder cancer and more recent observations concerning the induction of angiosarcomas in patients exposed to contrast material used for radiologic imaging studies and vinyl chloride exposure in the workplace in Louisville, Kentucky.[6]

ANATOMY OF COLON

The digestive tract is the system of organs within multicellular animals that takes in food, digests it to extract energy and nutrients and expels the remaining waste. The major functions of the GI tract are ingestion, absorption and defecation.

In a normal human adult male, GIT is approximately 6.5 meters (20 feet long) and consists of upper and lower GI tracts. The upper GI tract consists of the mouth, pharynx, oesophagus and stomach. The lower GI tract comprises the small intestine, large intestine and anus. A Large intestine is wider and shorter than the small intestine (approximately 1.5 metres in length as compared to 6.7 to 7.6 metres in length for the small intestine). The colon is 1.5 cm long and it itself is made up of caecum, the ascending colon, hepatic flexure, transverse colon, splenic flexure, descending colon and the sigmoid colon. The structural features are depicted in FIG. 1 and anatomical features of small intestine & large intestine are summarized in Table 1.

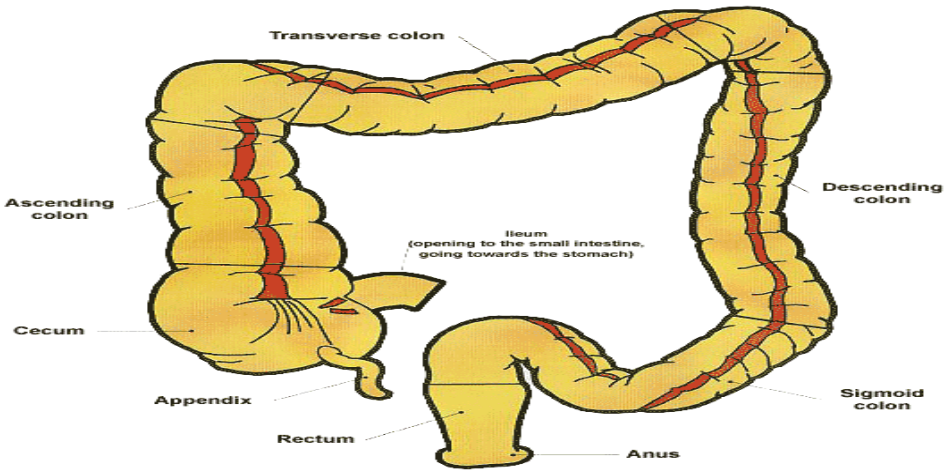

FIG. 1: Structural features of large intestine

Table 1: Anatomical features of small intestine and large intestine

Organ	Characteristics
Small Intestine	
Duodenum	It is 25-30 cm long section. It serves as a receiving area for chemicals and partially digested food from stomach.
Jejunum	It is about 40% of the small gut in man. It comes in contact with large number of intestinal cells containing thousands of tiny fingers like projections called villi which increases surface area to absorb most of the nutrients into blood.
Ileum	It is about 60% of the intestine in man. It contains goblet cells and peyer's patches. Here remaining nutrients are absorbed before moving into the large intestine.
Large Intestine	
Caecum	It is about 6-7 cm in length. It is the pouch where the large intestine begins. It is where ileum opens from one side and continues with the colon.
Ascending colon.	It is about 20 cm long. It is the part of the large intestine that goes from the bend on the right side below the liver and the caecum.
Hepatic flexure	It is on the right side of the body near the liver. It is the right angle bend in the colon that marks the connection of the ascending colon and transverse colon.
Transverse colon	It is about 45 cm long. It is the largest and most mobile part of the colon. It attaches the ascending colon to the descending colon by crossing the abdominal cavity. Its diameter varies from 9 cm in caecum to 2 cm in sigmoid colon; its average iameter is about 6.5 cm.
Descending colon	It is about 30 cm long. It traverses inferiorly along the left abdominal wall to the pelvic region.
Sigmoid colon	It is about 40 cm long. It is the part of the colon that forms an angle medially from the pelvis to form an S-shaped curve.
Rectum	It is about 12 cm in length. It is a short, muscular tube that forms the lowest portion of the large intestine and connects it to the anus. Faeces collects here until pressure on the rectal walls cause nerve impulses to pass to the brain, which then sends messages to the voluntary muscles in the anus to relax, permitting expulsion.

The colon serves following important functions:
1. Creation of suitable environment for the growth of colonic microorganisms.
2. Absorption of potassium and water from the lumen concentrating the faecal contents, secretion and excretion of potassium and bicarbonate ions.[7]
3. Through the muscular movements of colon faecal matter is pushed along until finally, the walls of the sigmoid colon contracts, causing the faeces to move into the rectum.
4. Synthesis of vitamin K by colonic bacteria promotes a valuable supplement to dietary sources and makes clinical vitamin K deficiency rare.

The colon is mainly situated in the abdomen; the rectum is primarily a pelvic organ. Further, the histological and microscopic structural evaluation of colon shows four layers: serosa, the mascularisexterna, the submucosa and the mucosa. The serosa is the external coat of the large intestine and consists of aerolar tissue that is covered by single layer of squamous mesothelial cells. The major muscularis coat of the large intestine is the muscularisexterna.

This is composed of an inner circular layer of fibers that surrounds the bowel. The submucosa is the layer of connective tissue that lies immediately beneath the mucosa. The colonic mucosa is further divided into three layers: the muscularis mucosae, the lamina propria and the epithelium.

COLON CANCER

The colon is an important part of the body's digestive system. The colon is a large muscular cylindrical tube (approximately five feet long) that collects and stores waste which then passes into the rectum. The principal functions of the colon are (1) absorption of water and electrolytes from the chyme to form solid feces and (2) storage of fecal matter until it can be expelled.

The colon is a 6-foot long muscular tube that connects the small intestine to the rectum. The large intestine is made up of the cecum, the ascending (right) colon, the transverse (across) colon, the descending (left) colon, and the sigmoid colon, which connects to the rectum. The appendix is a small tube attached to the cecum. The large intestine is a highly specialized organ that is responsible for processing waste so that emptying the bowels is easy and convenient.

Stool, or waste left over from the digestive process, is passed through the colon via peristalsis, first in a liquid state and ultimately in a solid form. As stool passes through the colon, water is removed. Stool is stored in the sigmoid (S-shaped) colon until a "mass movement" empties it into the rectum once or twice a day. It normally takes about 36 hours for stool to get through the colon. The stool itself is mostly food debris and bacteria. These bacteria perform several useful functions, such as synthesizing various vitamins, processing waste products and food particles, and protecting against harmful bacteria. When the descending colon becomes full of stool, or feces, it empties its contents into the rectum to begin the process of elimination (FIG. 2).

COLON CARCINOGENESIS: A MULTISTEP PROCESS

Colon cancer is one of the most common, best-understood neoplasms from a genetic point of view, yet it remains the leading cause of cancer-related mortality in men and women.[8] More than 1 million new cases of colorectal cancer (CRC) are diagnosed worldwide each year.[9] CRC is the 3rd most common malignancy and 4th most common cause of cancer mortality worldwide.[8] CRC is also the 2nd most common cause of cancer deaths in the United States and other developed countries, despite important advances in detection, surgery and chemotherapy.[9]

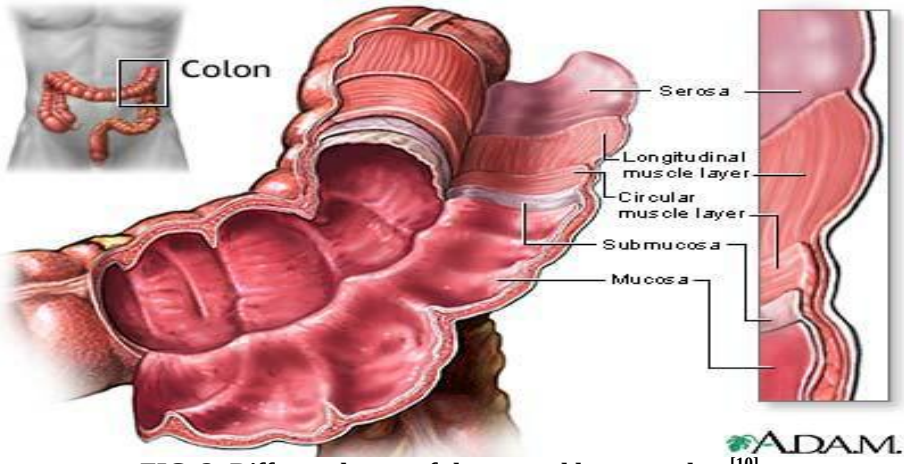

FIG. 2: Different layers of the normal human colon.[10]

In terms of epidemiological significance, colonic tumors are limited to hyperplastic polyps, adenomas and adenocarcinomas. Other types of benign and malignant tumors of the colon comprise of less than 5% of the tumor population.[11] Colonic hyperplastic polyps are frequently occurring lesion in the colon is the

hyperplastic polyp. If no cellular dysplasia is visible in the microscope, a polyp is scored as a hyperpastic polyp.[12] It is commonly believed that hyperplastic polyps, especially those located in the rectum and sigmoid colon are benign lesions lacking malignant potential. These polyps consist of alocalized zone of proliferation of intestinal mucosa associated with exaggerated cellular differentiation and maturation.

Colonic adenomas/adenomatous polyps are well demarcated lumps of epithelial dysplasia that can be classified into three major histological types: tubular, villous and tubule-villous. These are benign glandular neoplasms originating from intestinal mucosal epithelium characterized by incomplete cellular differentiation and by unrestricted cell division. The adenomas are classified according to the grade of dysplasia as mild, moderate or severe. Dysplasia predisposes an organ to cancer development. Adenomas are known to be the precursors of sporadic and hereditary colorectal cancer.[13] Most CRCs are adenocarcinomas which have developed from adenomas. Only about 5% of adenomas become malignant.[14] In sporadic cancer the progression from adenoma to cancer takes approximately 10-15 years.[15] Several studies have shown that removal of adenomas may reduce the incidence of colorectal cancer.[16,17,18] Adenocarcinomas of the colon are malignant tumors arising from the mucosal glandular epithelium. Abundant evidence from clinical and histopathological studies demonstrates that the majority of colon cancer arises from pre-existing adenomas over a long period of time. In fact, very often a continuous histological spectrum with increasing degree of atypia and progressive invasion can be seen in an adenoma containing a carcinoma.[19,20] This is often referred to as adenoma-carcinoma sequence.[21,22] On the other hands, there appears to be a little evidence to support an evolutionary relationship between hyperplastic and adenomatous polyps, thus hyper plastic polyps should not be included in the polyp-cancer sequence.[23]

Studies on colon cancer support the hypothesis that colon cancer progress through a multi-step process. A small benign adenomatous polyp may arise preferentially from instead of normal mucosa, the hyper proliferative mucosa or abnormal tissue architecture such as aberrant crypt foci (ACF).[24] Subsequent progression of a small adenoma to a large adenoma with increased malignant potential may occur in some cases. Finally, a fraction of larger adenomas may progress to invasive and metastatic cancer.[25]

These abnormal crypts can be identified by increased size, thicker epithelial lining and increased pericryptal zone after staining the colon with methylene blue.[26] But, recent reports has been demonstrated that adenomas arises from another preneoplastic lesions i.e., mucin depleted foci (MDF) which are formed by dysplastic crypts devoid of mucin.[27]

RISKS FACTORS FOR COLON CANCER

➢ Sporadic

Most cases of colorectal cancer are sporadic, and genetic and environmental factors are important. About 20% of all patients with this cancer are estimated to have some component of familial risk without fulfilling the strict criteria for hereditary colorectal cancer.[28] Family history should therefore always be taken when assessing a patient; the Bethesda guidelines are valuable in this context. However, taking a family history by interview often underestimates family history of colorectal cancer.[29] Most of the colon cancer cases are sporadic (88–94%) and the causes are older age, male sex, cholecystectomy, ureterocolic anastomosis, hormonal factors: nulliparity, late age at first pregnancy, early menopause. Environmental factors includes diet rich in meat and fat and poor in fibre, folate, and calcium, sendentary lifestyle, obesity, diabetes mellitus, smoking, previous irradiation, occupational hazards (eg, asbestos exposure) and high alcohol intake.

Personal history of sporadic tumours includes history of colorectal polyps, history of colorectal cancer, history of small bowel, endometrial, breast, or ovarian cancer.[30]

➢ Hereditary

Roughly 5–10% of all colorectal cancers develop in the setting of defined hereditary cancer syndromes. The two main forms are hereditary nonpolyposis colorectal cancer (HNPCC) and familial adenomatous polyposis (FAP).[29] Various hamartomatous polyposis syndromes are also associated with an increased risk of such cancer, such as Peutz-Jeghers syndrome, juvenile polyposis syndrome and Cowden syndrome.[28]

FAP is an autosomal-dominant disease. In about 80% of affected individuals, a germline mutation can be identified in the adenomatous polyposis coli (*APC*) gene. A subset of people with FAP and attenuated FAP has biallelic mutations of the *MYH* gene. FAP patients can develop more than 100 colorectal adenomas (50% of patients by age 15 years, 95% by age 35 years); if left untreated, colorectal cancer arises in almost all patients by age 40 years. Extra colonic manifestations, such as periampullary duodenal carcinoma (4–6% of patients) and desmoids (10–20% of patients) are a major cause of mortality and morbidity.

HNPCC is an autosomal-dominant disorder caused by germ line mutations of mismatch repair genes. Tumours that arise in the setting of HNPCC typically have a molecular characteristic called microsatellite instability, which helps in making the diagnosis. This instability is defined as frequent mutations in microsatellites, which are short repeated DNA sequences.

The penetrance of colorectal cancer in HNPCC is 70–85%. Risk is also increased for tumors of the genitourinary system, stomach, biliary system, pancreas,

small intestine, and CNS. A genotype-phenotype correlation was suggested for patients with HNPCC.[29]

MOLECULAR PATHWAYS INVOLVED IN COLORECTAL CANCER

A. CIN Pathway

a) The WNT Signalling Pathway

CIN is the most well characterized type of colorectal pathway and the most common. The tumorigenic process involves different mitotic spindle checkpoint regulators and proteins that mutually influence mitotic chromosome stability.[31] A "key" initial mutation is the early mutation of the adenomatous polyposis coli (APC) tumor suppressor gene, involved in both sporadic CIN and, when germline mutated, in all Familial Adenomatous Polyposis (FAP).[32] In FAP syndrome, an autosomal-dominant genetic disorder characterized by the development of hundreds to thousands adenomas in the colorectum during adolescence and young adulthood, there is a germline mutation of the APC gene that has been identified in 60% 80% of families with FAP.[33] An attenuated form of FAP (AFAP), characterized by less than 100 adenomas, occurs with APC germline mutations involving the 5' or 3' region of the gene. Importantly, 16%–40% of patients with less than 100 polyps carry the bi-allelic inactivation of the MUTYH based-excision repair gene, a condition called MUTYH-associated polyposis (MAP). Phenotypically AFAP and MAP are very similar.[34]

The APC tumor suppressor gene is involved in APC/β-catenin/Tcf pathway. Its inactivation results in increased WNT pathway signaling, through the failure to degrade β-catenin. The β-catenin cytoplasmic accumulation leads to its translocation into the nucleus and stimulates the TCF-targets, with increased proliferation, differentiation, migration and adhesion of colorectal cells. Mutations in genes implicated in APC/β-catenin/Tcf pathway in CRC lacking APC mutations are also found in sporadic CIN tumors, in particular mutations of β-catenin in 48% of tumors without APC mutations indicating that CTNNB1 mutations are present in the early stages of the colorectal pathogenesis and possibly substitutes the APC mutations in the stages of initiation.[35,36] Also, different components of the WNT/APC/β-cat pathway can be directly or indirectly altered—for example via constitutively activating β-catenin or Tcf. Among the various regulatory genes that interact with the APC suppressor gene, the mitotic checkpoint protein BubR1 was found to play a crucial role. BubR1 is a component of the mitotic checkpoint machinery along with Bub1, Bub3, Mad1, Mad2, Mad3, Mps-1 and CENP-E. By binding to Cdc20 it inhibits APC activity by stimulating a "wait anaphases" signal.[37] It's down regulation and consequent inactivation contribute to the formation of polyploid cells, prolonged cell survival, and excess proliferation, indicating a potential pathogenic mechanism in the initiation of chromosomal instability in CRC sporadic forms.[38]

The activity of β-catenin can be indirectly increased by mutations in oncogenes that regulate its activity at various levels. β-Catenin mutually interacts with different members of the Notch pathway, fundamental regulators of cellular differentiation and recently found involved in colorectal carcinogenesis.[39] Kwon and colleagues found that Notch1 increases the accumulation of active β-Catenin protein without needing ligand-receptor activation. They also found that the chronic use of non-steroidal anti-inflammatory drugs (NSAIDs), specifically Ibuprofen, induce a dose-dependent decrease of Notch pathway activity. This confirms the protective effects that have been extensively studied regarding the role of NSAIDs on colorectal cancer.[40]

Additional genetic perturbations that modulate β-Catenin activity include *CDK8* (cyclin dependent kinase-8) gene amplification, located at 13q12.13, that is present in approximately 60% of CRC cases. Increased CDK8 kinase activity acts as an oncogene in colorectal cancer by stimulating both β-Catenin[41] and Notch1, thus increasing transcription and cell differentiation (Fryer et al. 2004). Firestein *et al.* reported a significant association between *CDK8* expression and β-catenin activation, fatty acid synthase (FASN) overexpression and *p53* expression. *CDK8* over-expression was also significantly correlated with a poor CRC prognosis.[42]

Recently, activation of orphan receptors LGR-4 and LGR-5, G-protein-coupled receptors was found to increase signaling by binding with proteins in the R-respondin family, known potentiators of the WNT signaling pathway. The authors found that the increased Wnt/β-Catenin activity is obtained through enhanced WNT co-receptor LRP6 phosphorylation.[43]

Cyclin D1 (CCND1) was also found to be implicated in APC signaling. CCND1, together with other cyclin-dependent kinases that inhibit CCND1, such as *p27* (CDKN1B) and *p21* (CDKN1A), arecentral to cell cycle control especially in the transition from G1 to S phase (Alao 2007). Excessive CCND1 activation by APC mutation contributes to the development of colonic neoplasia by allowing the cell to escape apoptosis. Arber and colleagues evaluated the presence of CCND1 in normal colonic mucosa, adenoma and adenocarcinoma and confirm its increased expression only in mucosa from individuals affected by CRC.[44]

b) RAS Pathway

The above-mentioned early mutations of CIN pathway are then followed by subsequent events that promote new mutations and facilitate the tumor's progression from benign to malignant stages. The adenoma to carcinoma transition is determined firstly by the *K-ras*gene, a proto-oncogene that encodes for the GTPase protein involved in the transduction and propagation of extracellular signals—e.g., mitogen-activated protein kinase (MAPKs). Mutations of *K-ras*lead to a permanently active state that permits the cell to evade apoptosis and

acquire a growth advantage. More than 90% of mutations in the *K-ras*gene happen at codon 12 and at codon 13.[45] Mutations at codon 12 confer a more oncogenic phenotype than the mutations at codon 13, suggesting that codon 13 mutations are more involved in the adenoma-carcinoma transition; whereas codon 12 mutations predispose colorectal tumour cells to local invasion and metastasis.[46] Imamura and colleagues reinforced this hypothesis by confirming that the respective malignancy of the codon 12 and 13 mutations is independent of the *BRAF* mutations that are often associated with a poorer CRC prognosis: even after eliminating *BRAF* as a confounding factor, codon 12 mutations were implicated in significantly higher colorectal cancer specific mortality codon 13 mutations.[47] Studying the mutation in *K-ras*codon 12 and 13, in patients affected by CRC allows for the simultaneous evaluation of CRC prognosis and choice of chemotherapeutic strategies to pursue.

The RAS pathway is also involved with other signals critical for initiation of carcinogenesis. Horst and colleagues demonstrated that high WNT activity was connected with increased MAPK signaling, in *K-ras*mutated CRC samples.[48] Furthermore, Baba and colleagues saw an interaction between the AMP-activated protein kinase (AMPK) and MAPK. AMPK is a cellular energy balance status sensor, and plays a role in the regulation of cell proliferation and growth through the inhibition of the mTOR pathway and activation of the CDKN1A (*p21*) pathway and *p53*. Increased expression of phosphorylated-AMPK is associated with a good prognosis among p-ERK-activated CRC patients.[49]

c) The p53 System

p53 loss of function is frequently present in the later stages of colorectal tumorigenesis.[50] The *p53* gene is located on chromosome 17p and its mutation is one of the key steps in colorectal carcinogenesis and stimulates high proliferative activity through the loss of cell cycle control andapoptosis. Oikawa demonstrated that *p53* largely controlled *BubR1* transcription and expression and, in patients with 17p Loss of Heterozygosity (LOH), *BubR1* activity was significantly down regulated.[51] El-Deiry and colleagues identified a wild-type *p53*-activated fragment 1 (WAF-1), a highly induced gene (directly regulated by *p53*) that suppresses tumor cell growth in the *p53* pathway. When *p53* is mutated, the protective role of WAF-1 is not expressed. Another function of *p53* is to regulate energy balance, through activation of the AMPK pathway. Morikawa and colleagues further explored this role of *p53* in energy balance and described that among non-obese patients, *p53* positivity was associated with reduced cancer-specific survival while an adverse effect of obesity on CRC patient mortality was observed in *p53* negative subjects.[52]

During the progression of CRC pathogenesis, mutations in different cyclin-dependent kinases (CDKs) are also involved. P53, through the AMPK pathway, up-regulates the CDK inhibitor 1A (*CDKN1A* or *p21*), which is involved in regulating the cell cycle (energy balance status, cellular senescence and stem cell aging). Ogino and colleagues observed *p21* loss of function in 79% of CRC and found this to be significantly associated with *p53* expression. They demonstrated a positive correlation between *p21* loss and CRC survival with increasing patient age, specifically for patients >60 years. Moreover, the adverse effect of obesity in CRC is not observed in *p21* loss CRC.[53] Another CDK associated with *p53* mutations is with CDK inhibitor 1B (CDKN1B or *p27*).[54] P27 is involved in the control of the progression into S phase of the cell cycle and its degradation is associated with CRC progression.[55] CDKN1B expression is inversely associated with the MSI-H and CIMP-H types of CRC, and more in *p53*-negative cancers.

P53 also interacts with Cyclooxygenase-2 (COX-2), which plays a role in promoting inflammation and cell proliferation in CRC.[56] Interestingly, COX-2-positive tumors were found to be associated with an increased cancer-specific mortality regardless of *p53* status, indicating that *COX-2* could be an independent prognostic factor of colorectal cancers.[57]

B. MSI Pathway

The MSI pathway represents a form of genomic instability involved in the genesis of approximately 15% of sporadic colorectal cancer and >95% of Hereditary Non Polyposis Colorectal Cancer (HNPCC) syndrome. MSI is caused by the inactivity of the DNA Mismatch Repair (MMR) system. Disabled DNA MMR causes a 100-fold increase in the mutation rate in colorectal mucosa cells. The MMR system is a multi-protein system, which acts like a proofing machine to increase the fidelity of DNA replications by identifications and direct repair of mismatched nucleotides.[58] The MMR system acts only when an error eludes the intrinsic error checking system of DNA polymerase. In human cells, the functioning MMR system is composed of multiple interacting proteins including the human MutS homologue (MSH) 2 and human MutL homologue (MLH) 1.

CRC that develops through the MSI pathway presents peculiar clinical features: more often located in the proximal colon, with a poorly differentiated and a mucinous or medullary histo type and often presents intense peritumoral and intratumoral lymphocytic infiltrations.[59] In general, the prognosisand survival of patients affected by MSI-high CRC is better and longer than that of patients with CIN positive CRC. Importantly, MSI-high CRC does not respond to 5-fluorouracyl-based chemotherapies.[60]

In the HNPCC syndrome, CRC development is determined by germline mutation in one of the MMR components. HNPCC is an autosomal dominant genetic disorder characterized by a young age of onset (<50

years old) of colorectal cancer as well as other malignant tumors, including endometrial and ovarian cancers. In 95% of HNPCCs, mutations are present in *hMLH1* and *hMSH2*.[61] The clinical manifestations can be diverse, depending upon which gene is involved and where the mutations occur.[62] Defective *hMSH2* is associated with a 40%–60% increased risk of developing endometrial cancer, while defective *hMLH1* with a 50%–80% increased risk of developing CRC. Furthermore mutations in *hMSH6* are associated with 11%–19% increased risk of developing gastric cancer while mutations in *hPMS2* with a 9%–12% increased risk of develop ovarian cancer. Recently, a subclass of the MMR deficient HNPCC families have been found to carry germline deletions of the Epithelial Cell Adhesion Molecule (EpCAM) resulting in *hMSH2* gene silencing. EPCAM carriers show a lower risk of developing endometrial cancers. HNPCC is a good example of a genotype-phenotype association and the identification of mutation carriers is critical for implementing optimum screening and follow-up procedures. Also, in families with high suspected HNPCC, clinical parameters can help direct new suspected cases toward targeted genetic testing.

In sporadic settings, MSI-high CRCs are mostly due to epigenetic silencing of the *hMLH1* gene promoter. The resulting mutant phenotype, as in HNPCC settings, leads to inactivation of target genes, in particular tumor suppressors having a microsatellite sequence in their coding region. Importantly, sporadic MSI-high CRC cases harbor the *V600E* mutation of the *BRAF* oncogene, a member of the *RAF* family involved in the mediation of cellular response to the growth signal through the RAS-RAF-MAP kinase.[63] MSI-high sporadic CRCs display CIMP features (a combination of two pathways) and will be described further in the CIMP pathway section.

C. CIMP and the "Serrated" Pathway

A third pathway through which CRC progresses is the CpG island methylator phenotype (CIMP).[64,65] It consists of the aberrant hypermethylation of CpG di-nucleotide sequences localized in the promoter regions of genes involved in cell cycle regulation, apoptosis, angiogenesis, DNA repair, invasion and adhesion. The promoter hypermethylations cause the loss of gene expression. CIMP is found in approximately 20%–30% of CRC and it was reported that clinical features of CIMP CRCs are similar to those associated with MSI. An early event that is correlated with the progression of histological grades is the silencing of the *p16INK4a* tumor suppressor gene, whose loss of function causes uncontrolled cell proliferation, leading to neoplastic transformation.[66]

Based on the number of methylated markers, the CIMP phenotype can be also divided into CIMP-high and CIMP-low. The *BRAF* oncogene mutation is often identified in CIMP-high CRC and is associated with increased cell growth, progression of carcinogenesis, and high colon cancer specific mortality. However CIMP-high tumors, regardless of BRAF mutation, are associated with reduced colon cancer mortality.

Importantly BRAF V600E mutations were found in 90% of CRC cases with sessile serrated adenoma (SSA) lesions and never in the conventional adenomas. The BRAF mutation is an early event in the serrated pathway and its forced expression will lead to a state of dormancy known as senescence. In SSA, BRAF mutations were found either in early hyperplastic polyps (the serrated precursors) or in the advanced dysplastic serrated polyps, confirming its role in neoplastic progression.[67] The SSA polyps and the BRAF mutation frequently have CIMP-high and MSI-high features; thus, researchers established that, in sporadic settings, CIMP-high microsatellite unstable CRCs derive from the serrated pathway.[68]

BRAF and KRAS mutations are mutually exclusive.[69] Recently, researchers discovered that when KRAS mutation was found in CIMP CRCs, it is associated with lower markers of methylation, called CIMP-low. This is also frequently associated with mutations in the DNA repair gene Methylguanine Methyltransferase (MGMT) and with the loss of function of the PIK3CA. CIMP-low, in contrast with CIMP-high, appears to have different phenotype, with a low-level of DNA methylation. An alternative serrated pathway was extensively studied by Jass and colleagues, who described polyps in an "alternative serrated pathway", as a hybrid of adenomatous and serrated polyps. They hypothesized that these polyps, carrying *K-ras*mutation, represent only 2% of CRC, but present an extremely aggressive malignant potential, through in activations of MGMT.[70]

D. Other CRC Pathways

a) MicroRNA

Recently, microRNAs (miRNAs) have been found to be involved in CRC pathogenesis. miRNA are a class of short (20–22 nucleotide) non-coding RNAs which regulate protein expression by inhibiting mRNA translation, in particular of genes involved in cell differentiation, development, proliferation and apoptosis. The number of miRNAs involved in CRC pathogenesis is very large and still expanding, as new miRNAs are continuously being identified. They can be up regulated or down regulated in CRC, operating like oncogenes and tumor suppressor genes. For example, Bandres and colleagues found the altered expression of 13 miRNAs in patients affected by CRC and an interesting, divergent expression of miRNAs in CRCs with either KRAS or BRAF mutations indicating that these altered expressions may be related to miRNAs' regulatory action in the RAS pathway. Up regulation of *miR-31* was found to be associated with stage IV CRC.[70] Down regulation of *miR-145* and *miR-143* was demonstrated by other studies, showing that their expression is reduced in precancerous adenomatous polyps, as compared to

normal tissue; thus, researchers suggest these miRNAs play a key role in the early development of the tumors.

Significant up regulations of *miR-17-92*, *miR-17-5p*, *miR-20*, *miR25*, *miR-92-1*, *miR-92-2*, *miR-93-1* and *miR-106a* in the microsatellite stable (MSS) CRC and not in MSI CRC. Furthermore, demonstrated an increased expression of *miR-31*, *miR-183*, *miR-17-5p*, *miR18a*, *miR-20a* and *miR-92* in tumoral tissue as compared to normal colorectal mucosa, and saw an association between over expression of *miR-18a* and a worse CRC prognosis.[71]

Recently, high expression of *miR-203* was associated with poor survival among Caucasians with stage IV colorectal cancers: and interestingly, it was an indicator of poor survival in blacks with either stage I or II colorectal cancers. Finally, expression of *miR-21* expression predicted a poor prognosis in patients with stage IV cancer.[72]

b) Inflammatory Pathway

Chronic inflammation is a critical component of CRC initiation and progression. This is supported by finding of strong associations between IBD and CRC, and by findings supporting the positive effects of chronic NSAIDs use in CRC. Multiple different markers of inflammation predispose an individual to CRC. This happens by enhancing stimulation, by sustaining cell growth through promoting anti-apoptotic system and by increasing DNA-damage through the activation of the mutagenic reactive oxygen and nitrogen species. Other mechanisms include the production of angiogenic and lymphangiogenic growth factors, and changes of the membrane systems to facilitate invasion and altering cell adhesion.[73] In support of the role of chronic inflammation in CRCs, researchers studied the role of the pro-inflammatory cytokine tumor necrosis factor (TNF)-α, the transcription factor Signal Transducer and Activator of Transcription 3 (STAT3) protein, Interleukin (IL)-6 and the C-reactive protein (CRP). Chronically elevated levels of TNF-α promote tumor growth, proliferation and metastasis. IL-6 is a cytokine involved in the regulation of the acute phase of inflammation and, in its own transduction pathway, stimulates the transcription of STAT3 [74]. STAT3 activation stimulates its translocation into the nucleus and then stimulates cell proliferation, differentiation, apoptosis and promotes metastasis by inducing the expression of different gene targets—such as *VEGFR2* (vascular endothelial growth factor receptor 2), *Bcl-2*, *CyclinD1*, *MMP2-9*, *ICAM-1*, and *COX-2*.

CRP is a biomarker of inflammation, both in the acute phase and in the chronic low phase of inflammation. The role of this inflammatory mediator was controversial, as researchers obtained discordant results. Chan and colleagues, who investigated the influence of CRP, Interleukin-6 (IL-6) and Soluble Tumor Necrosis Factor Receptor 2 (TNFR-2, a TNF-α receptor super family member) in CRC, in a cohort of 33,000 women, found an increased risk of CRC in woman having high levels of sTNFR-2 (p = 0.03), but found no correlation with the other two markers. Interestingly, those with high baseline levels of sTNFR-2 who took aspirin had lower risk of developing colorectal cancer. On the other hand Song and colleagues researched the same inflammatory markers, and did not find any correlation, only a positive association between IL-6 and increased risk of CRC in lean individuals (p = 0.03). Moreover, Knupfer and colleagues found higher levels of IL-6 in neoplastic colorectal mucosa than in normal mucosa and strong associations between advanced CRC stage, tumor size and a worse prognosis.[75]

NATURAL COMPOUNDS IN PREVENTION OF COLON CANCER

The following compounds and plants have been identified with potent anticolon cancer activity. Bis-1,7-(2-hydroxyphenyl)-hepta-1,6-diene-3,5-dione,a bisdemethoxycurcumin analog (BDMCA)[76], Hesperetin(a citrus flavanone), Coconut cake[77], *Cynodondactylon(L.) pers*., Resveratrol[78], Ginger[79], Kaempferol[80], Luteolin, *Origanum Vulgarae L.*[81], Sibilin[82], Spices (Red chilli, Cumin, and Black pepper)[83], *Carumcarvi*[84], Gallic acid[85], Silibinin.[82]

Table 1: List of Medicinal plants for anti colorectal cancer with scientific validation

Scientific Name	Family	Part with extracted solvent	Experimental Model	Mechanism of Action
Aerva lanata	Amaranthaceae	Ethnolic aerial parts extract	Lung, Leukaemia, Prostate, Colon and Cervix cancer.	Inhibition of cellular levels of NADH and glucose levels.[86]
Ageratum conyzoides	Asteraceae	Kaempferol from ethylacetate leaf extract	Human non-small cell lung carcinoma (A-549), human colon adenocarcinoma (HT-29), human gastric carcinoma (SGC-7901), human golima (U-251), human breast carcinoma (MDA-MB-231), human prostate carcinoma (DU-145), human hepatic carcinoma (BEL-7402), and mouse leukemia (P-388) cancer cell lines.	Extract exhibited the highest cytotoxic activity on A-549 and P-388 cancer cells with IC50 values of 0.68 and 0.0003 µg/ml, Respectively. Extract containing Kaempferol rapidly scavenged DPPH at a concentration of 130.07 ± 17.36 g/kg.[87]

Amaranthus tricolor	*Amaranthacee*	Galactosyl diacylglycerols 1-3 from leaf and stem extract	Human AGS (gastric), CNS (central nervous system; SF-268), HCT-116 (colon), NCI-H460 (lung), and MCF-7 (breast) cancer cell lines	Compound 1 inhibited the growth of AGS, SF-268, HCT-116, NCI-H460 and MCF-7 tumor cell lines with IC50 values of 49.1, 71.8, 42.8, 62.5, and 39.2 mug/mL, respectively. For AGS, HCT-116, and MCF-7 tumor cell lines, the IC50 values of compounds 2 and 3 were 74.3, 71.3, and 58.7 microg/mL and 83.4, 73.1, and 85.4, respectively.[88]
Asparagus racemosus	*Liliaceae*	Ethyl acetate fraction of shatavarin IV from root extract	Human breast cancer, Human colon adenocarcinoma and Human kidney carcinoma of MCF-7, HT- 29 and A-498 cell lines.	Reduction in percent increase in body weight, tumor volume, packed cell volume, viable tumor cell count and increased non viable cell count and also restoration of hematological parameters towards normalcy.[89]
Carthamus tinctorius	*Asteraceae*	Dichloromethane, methanol and hexane flower extracts	Human colon cancer (SW 620 cell line)	Up regulation of Caspase 3, 7 and 9 and down regulation of Bcl2 transcripts. Stimulatory effect on The lymphocyte proliferation.[90]
Cassia fistula	*Fabaceae*	Rhein from hexane flower extract	Human colon cancer cell line COLO 320 DM	Cells treated with Rhein shoed the characters of apoptosis.[91]
Phyllanthus polyphyllus	*Euphorbiaceae*	Methanolic leaf extract	Ehrlich ascites carcinoma and human breast cancer (MCF7), colon cancer (HT29), and liver cancer (HepG2) cell lines	Decreases the levels of lipid peroxidation (LPO), glutathione peroxidase (GPx), glutathione Stransferase (GST) and increases the levels of superoxide dismutase (SOD) and catalase (CAT).[92]
Withania somnifera	*Solanaceae*	Aqueous extract of whole plant	Azoxymethane induced colon cancer in Swiss albino mice	Significantly altered the level of leucocytes, lymphocytes, neutrophils, immune complexes and immunoglobulins (Ig) A, G and M.[93]

CONCLUSION

Developed as well as developing countries cancer is a major public health burden. After the cardiovascular disease cancer is the second leading cause of death. Medicinal plants that have anticancer activity has role in treatment as well as chemo preventive purpose for colorectal cancer. For the neoplastic disease, mainly CRC has been emerged as a major challenge for mankind. For the treatment of CRC various drugs available in market (e.g. Capecitabine, Cetuximab, Trinotecan, etc.) and many others are under research. Marvelous possibilities are reviewed and collected from the natural treasure for the successful management of CRC.

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