CHAPTER 6

Discussion:

Plants, animals and minerals origin products become the main sources for the treatment of human diseases. WHO estimates that about 80% of world population in developing countries depends on traditional medicinal systems which are largely based on plants and animals for their survival. Leafy vegetables and herbs are used at least in part for the treatment or prevention of diseases because they are a good source of various minerals, vitamins, antioxidants and other medicinal compounds.

Amaranthus species are ancient cultivated crops; approximately 60 species are presently recognized. Although several species are often weeds. Green leafy vegetables are the sources that supply vitamins, minerals and trace elements hence they are consider as gold and also called as diet for poor man. Amaranthus is one among the 23 food plants that could be used to provide nutrition and enhance the quality of life of mankind\textsuperscript{140}. The amaranthus contain high protein, sulfur containing amino acids, several vitamins, carbohydrates, minerals and dietary fiber \textsuperscript{141}.

The whole plant of \textit{Amaranthus viridis}, \textit{Amaranthus caudatus} and \textit{Amaranthus spinosus} were collected from the surrounding of Chickballapur and were authenticated.
*Amaranthus* *spinosus*, commonly known as “Mulluharivesoppu” in Kannada. In Indian medicinal system *A. spinosus* is used as analgesic, antipyretic, laxative, diuretic, digestible, antidiabetic, anti-snake venum, antileprotic, blood diseases, bronchitis, piles and anti-gonorrheal\(^{32,33,34}\). Some tribes in India apply *A. spinosus* to induce abortion\(^{35}\), its juice used to prevent swelling around stomach and while the leaves are used to cure jaundice \(^{36}\).

*Amaranthus caudatus* Linn, commonly known as “Peddathotakura” in Telugu. In India *A. caudatus* traditionally used in curing kidney stones, stomach ache, piles, leprosy and fever\(^{37}\), as diuretic, vermifuge, blood purifier and astringent\(^{38,39}\). Seeds of *A. caudatus* used to treat amoebiasis and jaundice in Southeastern Ethiopia\(^{40,41}\). *A. caudatus* leaf tea used for relieving pulmonary conditions\(^{42}\) and as an abortifacient \(^{43,44}\).

*Amaranthus viridis* Linn called as ‘Chilaka thotakura’ in Telugu. Indian and Nepalese traditional system used *A. viridis* to reduce labour pain and as antipyretic\(^{32,45}\). The bruised leaves directly applied to eczema, psoriasis and rashes by Negritos of the Philippines\(^{42}\). Other traditional uses; anti-inflammatory agent of the urinary tract, vermifuge, laxative, diuretic, antiulcer, analgesic, antiemetic, venereal diseases improvement of appetite, antileprotic, and for treatment of respiratory disorders \(^{32,46,47-53}\).
It is essential that standards have to be documented to control and ensure the identity of the plant and determine its quality prior to use. A comprehensive pharmacognostic evaluation therefore is highly necessary requirements. According to World Health Organization (WHO) prove for identity and purity, criteria such physical constants; moisture, ash content, extractive values adulteration, and macroscoa and microscopical examinations are first step\textsuperscript{143,144}.

In case of incorrect labeling of botanical quality can be a problem. Paraguay tea is South American product was linked with an outbreak of anticholinergic poisoning in New York. Chemical analysis revealed that the chemical constituents present, are different from the metabolites usually found in the plant from which Paraguay tea is made\textsuperscript{145}.

Ash values are useful in evaluating the crude drugs quality and purity. The total ash contains of sodium, potassium, magnesium and calcium salts of carbonates, phosphates, and silicates\textsuperscript{139}. The total ash value of \textit{A. viridis} was found to be lesser than \textit{A. spinosus} and \textit{A. caudatus}. The acid insoluble ash and water-soluble ash were more in \textit{A. spinosus} than \textit{A. caudatus} and \textit{A. viridis} respectively.

Extractive values are helpful for evaluation the nature of chemical constituents and its yield in particular solvent. The
amount of extractive yield to a given solvent is often an estimated measure of a definite constituents or group of related chemical constituents present in the plant. The menstrum used for drawing out should be able to dissolve maximum amount of desired phytoconstituents. Water extractive value for the three selected plants of Amaranthus showed more compared to the ethanol extractive values.

Microscopic evaluation showed the presence of glandular trichomes in A. spinosus while A. caudatus and A. viridis found to contain covering trichomes, as a differentiating character while other anatomical features appeared to be same.

Preliminary phytochemical study of methanol extract of A. spinosus, A. caudatus and A. viridis showed the presence of alkaloids, steroids, cardiac glycosides, anthraquinone glycosides, phenolic compounds, tannins, saponins, proteins and carbohydrates. Fingerprint is an important parameter in the standardization and evaluation of herbal material. It offers a more or less exact picture of the detectable phytoconstituents in a particular solvent system. We adopted a solvent system in the mid polar region where many flavonoids and phenolic compounds show up. Since the plants are known to contain flavonoids and some of the pharmacological activities can be attributed to the antioxidant properties of the plants. Many different solvent systems tried for
the finger print like, Ethyl acetate: formic: water (4:1:0.5), Toluene: Ethyl acetate: Acetic Acid: water (5:3:1:2) and so on, Toluene: Ethyl methyl Kenton (9:1) gave very good resolution with maximum number of peaks at 350 nm. Different wavelengths were tested, 205nm gave large number of peaks but noise level appeared to be too high, moreover at this wavelength even impurities give absorption, therefore discarded. At 350 nm, clean and opt peaks were obtained. Once again scanning was done at 340, 350 and 360 nm with narrow window to find if more peaks could be achieved. These wave lengths did not show much variation and hence peaks at 350 nm are shown below. These peaks can be used as standards for the future reference. Methanol extracts of amaranthus contain flavonoids (rutin and quercetin) and MeAc and MeAs with especially better antioxidant than MeAv according to the HPTLC report. Rutin and Quercetin content in the methanol extracts of each plant was estimated by HPLC. All the three plants showed a small difference in percentage of Rutin and Quercetin content.

Antioxidant compounds in plants (vegetable, medicinal herbs etc) play an important role as a health-protecting factor. Herbs contain wide variety of phytoconstituents like phenolic compounds (coumarins, falvonoids, tannis, phenolic acid), vitamins (ascorbic acid, vitamin E), and nitrogen compounds (proteins, aminoacids,
alkaloids, betalains, beta catotene and some endogenous metabolites are having strong antioxidant potential. These phytoconstituents have shown to possess anti-inflammatory, anticancer, antiatherosclerotic, antimicrobial, antiviral, reduced cardiovascular disease, LDL-cholesterol, diabetes and as anti-ageing agents.

The main attribute of an antioxidant is its capacity to trap free radicals. Biological systems contain oxygen species and reactive free radicals from a wide variety of sources. Nucleic acids, proteins, lipids or DNA are oxidized by free radicals and causes chronic diseases. Antioxidants inhibit the oxidative mechanisms that causes to chronic diseases by scavenge reactive oxygen species\textsuperscript{146}. Number of clinical investigations signifying that a fruit, vegetables, tea and red wine contains antioxidants; are the main factors responsible for reducing the occurrence of chronic diseases like cancer and heart disease. Many synthetic drugs defend against oxidative damage but they have undesirable side effects. These side effects can be overcome by consuming food supplements and herbal drugs because of its antioxidant properties\textsuperscript{147,148}. Several natural antioxidants have been isolated recently from herbs\textsuperscript{149-151}.

It has been recommended that every day up to 1 g of polyphenolic compounds ingestion protect humans from chronic diseases. This activity is due to their redox properties\textsuperscript{152}, which
play a vital role in absorbing and neutralizing free radicals or decomposition of peroxides. Polyphenols were reported to eradicate radicals due to their hydroxyl groups, and they contribute directly to antioxidant effect and it has an important role in stabilizing lipid oxidation\textsuperscript{153}. The total phenolic content was found to be higher in methanol extract of \textit{A. caudatus} compared to methanol extract of \textit{A.spinokus} and \textit{A. viridis}.

The antioxidant effects on DPPH are due to their hydrogen donating ability\textsuperscript{154}. Free radicals are major factor in biological damages and DPPH has been used to assess the free radical scavenging activity of natural antioxidants\textsuperscript{155}. DPPH; which is a radical itself with a purple colour, changes in to a stable compound with a yellow colour by reacting with an antioxidant and the extent of the reaction depends on the hydrogen donating capacity of the antioxidant \textsuperscript{156}. The declining capability of the DPPH radical is determined by the reducing in its absorbance at 510 nm, due antioxidants. Results revealed that methanolic extract of \textit{A. caudatus} has the better DPPH scavenging activity than \textit{A.spinokus} and \textit{A.viridis}.

Nitric oxide plays an important role in different inflammatory processes. A constant level of production of Nitric oxide radicals are directly lethal to tissues and contribute to the vascular subside connected with septic upset and also connected with various
cancers and inflammatory conditions including juvenile diabetes, arthritis, multiple sclerosis, and ulcerative colitis. The nitric oxide toxicity increases considerably when it interact with superoxide radical, leads to highly reactive peroxynitrite anion (ONOO\textsuperscript{-}). Nitrite formation was inhibited by extracts in the reaction with nitric oxide and oxygen. The present study proved that methanolic extract of *A. caudatus* has the potent nitric oxide scavenging activity than *A. spinosus* and *A. viridis*.

Mann and Keilin, first isolated Superoxide dismutase (SOD) from bovine blood in 1938 and McCord and Fridovich discovered its catalytic function in 1969. Superoxide dismutase plays important role in the safeguard of cells against oxidative damage. SOD catalyzes into hydrogen peroxide and oxygen. SOD is an endogenously produced intracellular enzyme, present in the body. There are three types of SOD; CuZn-SOD has been found in eukaryotic cells, Fe-SOD has been observed in prokaryotic cells and Mn-SOD has been found in both the type of cells. The Copper ion in the enzyme is responsible for the redox property where as the Zinc ion responsible for stabilization of enzyme. A copper/zinc SOD, isolated from beef liver, has been used for degenerative joint disorders as an anti-inflammatory agent. Superoxide radical is involved in many chronic conditions like inflammatory tissue injuries in arthritis, ischaemia reperfusion, and gastric ulceration.
In this assay, superoxide ions (O$_2^-$) are produced by Phenazine Methosulphate/beta-Nicotinamide Adenine Dinucleotid, which converts of NBT to NBT-diformazan, which shows absorption at 560 nm. SOD declines the superoxide ion concentration and reduces the rate of NBT-diformazan formation. NBT-diformazan appearance declines, which determines the SOD activity of the plant extracts\textsuperscript{159}. Methanolic extract of \textit{A.caudatus} possess strong superoxide radical scavenging activity than \textit{A.spinosus} and \textit{A.viridis}.

In ABTS assay, ABTS reacts and potassium persulphate to produce a blue green chromogen ABTS$^{-}$ radical cation. In the presence of the antioxidants, the colored radical cation is converted back to colorless \textsuperscript{160}. In the present study, the methanolic extract of \textit{A.caudatus} showed significantly higher scavenging of ABTS$^{-}$ radicals when compare to \textit{A.spinosus} and \textit{A.viridis}, representing that they may be valuable therapeutic agents for treating radical related pathological damage.

Highly reactive tissue damage species (hydroxyl radical) were formed by the interaction of Fe$^{2+}$,$^{3+}$ with hydrogen peroxide in living systems \textsuperscript{161,162}. Various reactive iron-oxygen complexes may also exist, such as ferryl, perferryl and Fe$^{2+}$/Fe$^{3+}$/O$_2$ species\textsuperscript{163,164}. The pentose sugar 2-deoxyribose is attacked by *OH radicals to yield a mixture of products \textsuperscript{165}. On heating with thiobarbituric acid at low
pH, these products react to form a pink chromogen that can be measured by its absorbance at 532 nm; this chromogen is indistinguishable from a thiobarbituric acid-malondialdehyde (TBA-MDA) adduct\textsuperscript{166}. Production of a TBA-MDA adduct obtained from deoxyribose, and is a simple test for OH formation in biological systems, if appropriate organize experiments are performed\textsuperscript{166,167}. The results revealed that methanolic extract of \textit{A.caudatus} showed higher scavenging of hydroxyl radicals when compare to \textit{A.spinosus} and \textit{A.viridis}.

Wohlegemuth first introduced the measurement of amylase quantitatively by iodometric method\textsuperscript{168}. Procedure for standardizing the quantity of starch and iodine was given by Somogy\textsuperscript{169}. A dye-coupled starch method was introduced by Rinderknecht \textit{et al.}, which was very simple to carry out. Turbidimetric measures are relatively fast but it requires special instruments and has difficulty in producing reproducible starch solutions\textsuperscript{170,171}. Numerous enzymatic procedures have been recommended\textsuperscript{172}, including one that used a substrate maltotetrose\textsuperscript{173}. These methods represented major improvement in amylase measurement\textsuperscript{174}.

Alpha amylase inhibition was studied by using CNPG-3 method. CNPG-3 is 2-chloro-p-nitrophenol linked with maltotriose, a chromogenic substrate. The amylase reacts with
CNPG-3 results in the formation of 2-chloro-p-nitorphenol, which can be measured spectrophotometrically at 405 nm. This reaction proceeds very rapidly; α-amylase hydrolyzes the CNPG-3 to release 2-chloro-nitrophenol and form 2-chloro-p-nitrophenyl-α-D-maltoside (CNPG2), Maltotriose (G3) and glucose (G). Herbal drugs that inhibit carbohydrate-hydrolyzing enzymes have been established to diminish postprandial hyperglycemia and without promoting insulin secretion in NIDDM patients, improves impaired glucose metabolism. These herbal drugs are useful for patients taking oral antidiabetic agents who require a supplementary medication to maintain their blood glucose levels within a safe range. Our studies demonstrate an appreciable α-amylase inhibitory activity with the all three methanol extracts. Methanolic extract of A.caudatus showed potent alpha-amylase inhibitory activity compared to A. spinosus and A.viridis.

International expert committee has recently proposed a amendment of diagnostic criteria for diabetes, recommending that glycosylated haemoglobin (HbA₁C) may be a improved means of diagnosing diabetes than measures of glucose and hence it should be adopted as a diagnostic measure for diabetes. Glycation of hemoglobin has been associated with diabetes mellitus. Monitoring the HbA₁C in diabetic patients may improve treatment. Red blood cell live for 8-12 weeks, glucose molecules join hemoglobin and
forming HbA₁C. In poorly controlled diabetic patients, increases in the quantities of the HbA₁C are noted. Methanol extracts of amaranthus showed potent hemoglycosylation activity and may be useful for treating diabetes.

Methanol extracts of *A.spinosus*, *A.caudatus* and *A.viridis* at single dose of 2000 mg/kg did produce neither behavior changes nor mortality in rats and mice when observed for 14 days after administration.

Analgesic drugs commonly determine nociception and involve testing the animal reaction to painful stimuli. It is possible to assume that certain noxious stimuli (thermal, mechanical or chemical) are painful and that reflex movements or behaviours resulting from such stimuli are indicative of a pain threshold. Hence the use of the three antinociceptive models: Acetic acid induced writing reflex, Hot plate, Tail immersion models for the study of the analgesic activities of MeAs, MeAc and MeAv.

Several chemicals like acetic acid and phenylquinone induces writhings in laboratory animals. Intraperitoneal administration of acetic acid in this experiment produced abdominal writhing by stimulating the chemosensitive nociceptors in the laboratory animals. The percentage decrease in the number of abdominal writhings indicates the level of analgesia in
the acetic acid induced writhing reaction model\textsuperscript{185}. The methanolic extracts of amaranthus exhibited significant ($P<0.01$) analgesic activity by reducing the acetic acid-induced writhing responses. Methanolic extracts at 200 and 400 mg/kg dose produced analgesic effect that was comparable to reference drug diclofenac sodium the reference drug.

The hot plate and tail immersion are the most general tests to assess the central analgesic property of drug or extract. Hot plate and tail immersion methods are further useful in distinguishing by their affinity to the pain stimuli through mechanism of neuronal pathways, in hot plate method mechanism involves brain functions and is a spinally-organized reaction and in tail immersion method mechanism involves spinal reflex to nociceptive stimuli, whereas the \textsuperscript{186}. Narcotic analgesics inhibit both peripheral and central mechanism of pain, whereas non-steroidal anti-inflammatory drugs inhibit only peripheral pain\textsuperscript{187,188}. Methanol extracts of amaranthus at 200 and 400 mg/ml dose of showed significant analgesic activity ($P<0.01$), which was comparable to the effect of standard Morphine 5 mg/kg. Our studies revealed that methanolic extracts of amaranthus established significant analgesic activity, by acting through peripheral and central pain mechanism.
Pyrexia is caused as a resulting impact of infection, tissue damage, inflammation, or other diseased situation. Pyrexia is a natural defense mechanism of body to create an environment to destroy infectious agent. Generally the infected or damaged tissue provoke the development of pro-inflammatory mediators like cytokines like interferons, lymphokines, interleukin and Tumor necrosis factor alpha, which enhance the production of prostaglandin E$_2$ (PGE$_2$) and triggers hypothalamus to raise the body temperature$^{189}$. Temperature regulatory system is governed by a nervous response mechanism, when the body temperature is extremely high it dilates the blood vessels and increase sweating to decrease the temperature. High fever frequently increases earlier disease progression by rising tissue catabolism, dehydration and as in HIV$^{190}$. Most of the antipyretic agents reduce the increased body temperature by by inhibiting COX-2 expression and PGE$_2$ biosynthesis$^{191}$. Synthetic antipyretic agents selectively inhibit COX-2, but are lethal to the glomeruli, hepatic cells, brain and heart, whereas natural drugs are of lower selectivity to inhibit COX-2, with less side effects$^{192}$. Recently herbal drugs with potent antipyretic activity achieve recognized impulsion as the accessible antipyretics, such as paracetamol, nimusulide etc, which have lethal cause to the body$^{193}$. Our results revealed that methanol extract of three plants of amaranthus possesses a significant
(P<0.01) antipyretic effect in reducing yeast-induced elevated body temperature in rats and their effects were comparable to that of the standard drug paracetamol.

Constipation affects commonly 24% worldwide, North American adult 15% of the population\textsuperscript{194}. It is more frequent than several other chronic diseases, including migraine, obesity, hypertension, and diabetes\textsuperscript{195-199}. Women were more prone to constipation more than men. Constipation occurs in all age groups but is more common in older than 65 years and younger than 4 years \textsuperscript{200,201}. Constipation financial records for more than 2.6 million office visits and more than $600 million spent on laxatives every year \textsuperscript{202,203}. Peoples with constipation also may report decreased productivity and increased absenteeism.\textsuperscript{205} Physicians primarily define constipation on the basis of stool frequency, considering less than three bowel movements per week to be abnormal\textsuperscript{197}. In contrast, patients typically define it on the basis of inconvenient symptoms such as straining, passage of hard stool, unproductive urges, lack of ability to defecate at will, and sensations of incomplete evacuation \textsuperscript{206}.

Constipation can also be caused by the side effects of drug particularly antispasmodics, analgesics, antidepressants, antipsychotics, sedative, anticholinergics, antihypertensives and
cation containing agents such as iron preparation and aluminium (antacids)\textsuperscript{207}.

Senna is the most commonly used anthrquinone drug, because of the presence of laxative constituents like Sennoside A, B, C and D. Anthrquinones stimulate intestinal peristaltic movement and initiate retention of fluid in the intestinal lumen, and increased electrolyte and water transportation in the colon are vital modes of action of anthraquinones. Mechanism of action of anthraquinones: effect on large intestine motility resulting in accelerated colonic transit, thereby reducing absorption fluid and effect on secretion process causing enhanced fluid absorption. Anthraquinones may also concerned in regulation of transintestinal electrolyte and water movements comprise the energy providing Na\textsuperscript{+}/K\textsuperscript{+}ATP ase, the mediators of membrane permeability and may also inhibit prostaglandins\textsuperscript{208}.

A neurotransmitter released from parasympathetic nervous system is acetylcholine and which shows effect on gut by stimulation of muscarinic receptors subtypes (M\textsubscript{3}) and leads to regulate the peristaltic movements of GIT\textsuperscript{209}. Methanol extract of A. spinosus, A. caudatus and A. viridis significantly (\textit{P}<0.01) increased propulsion of the charcoal meal through the gastrointestinal tract at 100 mg/kg dose comparable to Senna used as standard. Methanol extracts of three plants potentiates the concentration
response curve of acetylcholine on isolated rat ileum. *A. caudatus* showed maximum contraction response when compared to *A. viridis* and *A. spinosus*. Methanol extracts at dose of 100 mg/kg (p.o.) significantly increased (*P*<0.01) fecal output of rats when compared to control group. The present study suggestes that methanol extracts of amaranthus possess stimulant effect on gastrointestinal tract.

Helminthic infections of the gastrointestinal tract of human and animals have been documented to have adverse effect on health standards. Various plants were used for screened their anthelmintic activity using different species of worms. But earthworms have been commonly used for the preliminary evaluation of anthelmintic activity by *in vitro*, because earthworms similar to intestinal worms and their response to anthelmintic agents and are available easily. Substances toxic to the earthworms are valuable for investigation as an anthelmintics. Earthworms have the capability to move by ciliary movement. The outer layer of the earthworms is a mucilaginous in nature and composed with complex polysaccharides. The outer layer enables the earthworms to move freely. Any injure to the mucopolysaccharide membrane leads to paralysis and may leads to the death of the worms. Methanol extracts showed significant (*P*<0.01) antihelmintic activity. The results of present study
indicates that methanol extracts exhibits dose-dependent anthelmintic activity it could be effective against parasitic infections of humans.

All organisms need to protect against microorganisms and prevent the onset of infection is believed to depend on their innate immune response. Some plant based biologically active compounds of plants have been explored for the growth inhibition of pathogenic microbes because of their antimicrobial potential\textsuperscript{211}. The problem of resistance to microorganisms is growing and the use of antimicrobial drugs in the future is still uncertain. So the development of new families of antibiotics that can overcome the resistance problem has become very important\textsuperscript{212}. The main goal is to provide capable antimicrobial drugs to the patients. Thus, natural antimicrobial agents are an attractive option for their greater efficacy and lower cost\textsuperscript{213}. The microorganisms selected for this study were \textit{Staphylococcus aureus}, \textit{Streptococcus}, \textit{K. pneumonia}, \textit{B. subtilis} and \textit{E. coli} by disc diffusion method. MeAc shows potent antimicrobial activity compared to MeAs and MeAv.

\textit{A. spinosus}, \textit{A. caudatus}, and \textit{A. viridis} contains amino acids namely, lysine, arginine, histidine, cystine, phenylalanine, leucine, isoleucine, valine, threonine, methionine, tyrosine and tryptophan\textsuperscript{214}. These amino acids contribute positively to the antioxidant activity\textsuperscript{215-220}. Amaranthus also reported to contain
beta-carotene, thiamine, riboflavin, niacin and ascorbic acid\textsuperscript{221}. Carotenoids serve as precursors of vitamin A, which shows antioxidant activity\textsuperscript{222}. Ascorbic acid acts as a scavenger for free radicals formed during metabolic process\textsuperscript{223}. Niacin is needed for proper digestion and healthy nervous system. These B vitamins help the body to metabolize fats and protein.

The phytochemical screening of methanol extract of amaranthus revealed the presence of flavonoids (rutin and quercetin), tannins, steroids, saponins, cardiac glycosides and amino acids have been reported to have various biological effects such as antioxidant activity\textsuperscript{224}, analgesic\textsuperscript{225}, anti-inflammatory\textsuperscript{226}, antiulcerogenic\textsuperscript{227}, cytotoxic, antihypertensive, hypolipidemic, antiplatelet, neurodegenerative diseases\textsuperscript{228}, antiallergic and inhibitory action on arachidonic acid metabolism as demonstrated by \textit{in vitro} and \textit{in vivo} tests\textsuperscript{229}. There are also reports on analgesic effects of alklaoids\textsuperscript{221}, sterols and triterpenoids\textsuperscript{230}.

For a long period, herbal drugs comprise an important source for management of human health. \textit{Amaranthus spinosus}, \textit{Amaranthus caudatus} and \textit{Amaranthus viridis} exhibits antioxidant, analgesic, antipyretic, anthelmintic, antimicrobial, inhibition of alpha amylase and enzymatic haemoglycosylation, spasmogenic and laxative pro pertied. Therefore, our results provides scientific data for the ethnomedicinal claims