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Editorial

The key to medicinal plants research revolves around the detection, isolation, and characterisation of antioxidants as therapeutic agents

Plant-derived products are potential sources of pharmaceutical industry. So since the ancient period to modern man every body at every moment searches for a new and effective method for value added products and plants with medicinal values. In this issue much work on the identification of active ingredients responsible for pharmaceutical properties of medicinal plants are reported using animal model to prove their efficacy.

Indian Ayurveda used indigenous plants as contraceptives. They act either by preventing implantation or by suppressing spermatogenesis (Breuner, 2005). Still an alternative to hormonal pill is not yet available, although attempts are made to develop safe herbal contraceptives. In West Bengal tribal people use the leaf extract of *A.egle marmelos* (Bael) for contraception of male (Bhattacharya, 1982). *A. marmelos* is a tree (family of Rutaceae) with almost all the parts - roots, leaves, fruits and barks have a medicinal value (Duke and Jo, 2002, Das et al., 2006). Das et al (2009, in this issue) studied antigonadal activity of hydro-methanolic extract of leaf of *Aegle marmelos* using male albino rats. Sperm count, sperm viability, sperm motility, plasma testosterone level and androgenic key enzyme activities in testis were studied. The focus was on the effect through hypothalamico-hypophysial axis for the induction of antigonadal activity to develop a herbal male contraceptive in future. Results of this experiment show that the hydromethanolic extract of the leaf has the antitesticular activity and inhibited pituitary gonadotropin secretion, acting on testicular tissue and/or sperm cells for induction of oxidative injury that may suppress the testicular steroidogenic and gametogenic activities. The active ingredient responsible for such action is to be isolated and characterized in further work.

In an elegant survey, Borchardt et al. (2009, in this issue) studied seeds from 158 plant species of the Mississippi River Basin of the USA and evaluated these seeds as potential new sources of antimicrobial and antioxidant activity for use in pharmaceutical and nutritional purposes. Leaves, roots, flowers, whole plants, and stems are commonly screened for useful phytochemicals, with very few reports on seeds. But, a wide range of chemical compounds are present in seeds including alkaloids, lectins, and phenolic compounds such as lactones, tannins and flavonoids. These compounds protect seeds from microbial degradation. So in this study the authors inventorised the antioxidant and antimicrobial activity of seed extracts from native and naturalized plants of the Mississippi River Basin. Antioxidant levels of these plants ranged from 2,400 µM Trolox/100 gm (TE) to 261,384 TE. Extracts of seeds from 35 species had antimicrobial activity. The plants with high antioxidant values were not necessarily with effective antimicrobial activity. Further studies are needed for the temporal and spatial variations in plant antioxidant and antimicrobial activity in relation to the time of harvest, seed storage conditions and extraction methods. Seasonal changes, environmental factors and stage of plant development effect the production and distribution of bioactive constituents in the plant. With these challenges, screening of native may lead to the development of new products for use as nutritional (antioxidants) and pharmaceutical agents. Such an inventorisation of local plants will be economically and medicinally helpful in the future.

Adesanwo et al. (2009, in this issue) from Nigeria made a brief study on the active principle of *Melaleuca bracteata* F, Muell (family Myrtaceae, the South African tea tree or Johannesburg gold is an ornamental plant) which is responsible for its medicinal use. The leaf is useful in curing wounds, skin disorders, stimulates glandular secretions, and reduces vein congestion. The plant is used by Zulus in South Africa as a anti-HIV agent (Hutchings et al., 1996). Oils of the leaves of Melaleuca species (methyl eugenol from M. bracteata; 1, 8-cineole from M. quinquenervia and terpinen-4-ol from M. armillaris) are antimicrobial (Wilkinson and Cavanagh, 2005). But the plant is not known to have any gastro protective and anti-ulcer agent. Isolation of BA in the extract actually prompted the assay. Ethyl acetate extract of *Melaleuca bracteata* reduced gastric acid secretion and indomethacin-induced ulceration. This study also reports the isolation of 3-hydroxyl-lup-20 (30)-ene- 28-oic (betulinic acid, BA), 3-hydroxylolean-12-ene-28-oic (oleanolic acid, OA) and its acetate from M. bracteata extract. The presence of BA and OA in the extract may account for its anti-ulcer effect.

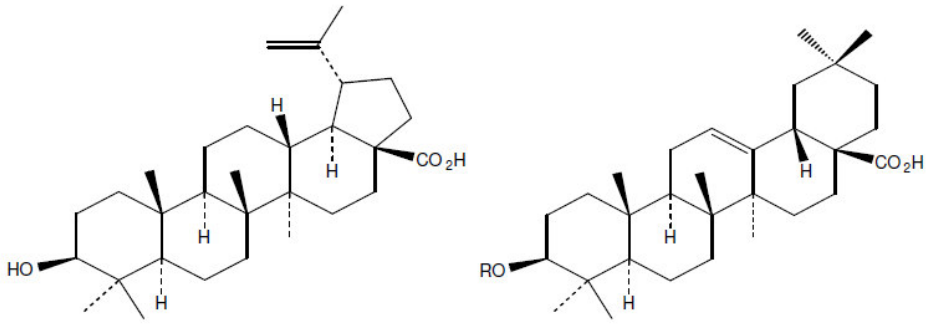

Figure 1. [1] betulinic acid [2] oleanolic acid (R=H) and [3] oleanolic acid acetate(R = $COCH_3$)

These two principles are reported to have gastroprotective effects, anti-inflammatory, antitumour, anti-oxidant, anti-diabetogenic, anti-HIV (Somova et al., 2003). The antiulcer effect observed in this extract might therefore be due to inhibition of gastric acid. These results therefore seem to suggest that betulinic, and or oleaolic acid containing materials may have potential antisecretory effect. Future studies are needed to elucidate the mode of action of the extract and its constituents (Adesanwo et al. 2009, in this issue).

In a similar but exhaustive analysis Apea-Bah et al. (2009, in this issue) studied gambir (*Uncaria gambir* L. Family Rubiaceae) is used in traditional medicines such as treatment of wound and ulcer, fever, headache, gastrointestinal illnesse and bacterial and fungal infections. The aqueous extracts of leaves and young twigs of *U. gambir* are used for the treatment of diarrhea and dysentery, and used as gargle for treatment of sore throat (Taniguchi et al., 2007). Dried hooks are used as spasmolytics, analgesics and sedatives for symptoms associated with nervous disorders and in the treatment of hypertension (Heitzman et al., 2005). The antioxidant properties are attributed to the presence of polyphenols like tannins, catechin, gambiriins (Taniguchi et al., 2007). Pine bark extract which is rich in polyphenols such as catechin, quercetin, dihyhydroquercetin, taxifolin and phenolic acids, is reported to be effective in suppressing postprandial hyperglycemia in diabetics (Kim et al., 2005). Even though gambir is also rich in polyphenols, it is not used traditionally in treating diabetes mellitus. There is the need to study its potential as an inhibitor of glucosidase. In the present study, we investigated the free radical scavenging and glucosidase inhibitory potential of commercial gambier sold in a local Indonesian market. The major compound responsible for the bioactivity was qualitatively identified using reverse phase HPLC, LCMS and NMR.

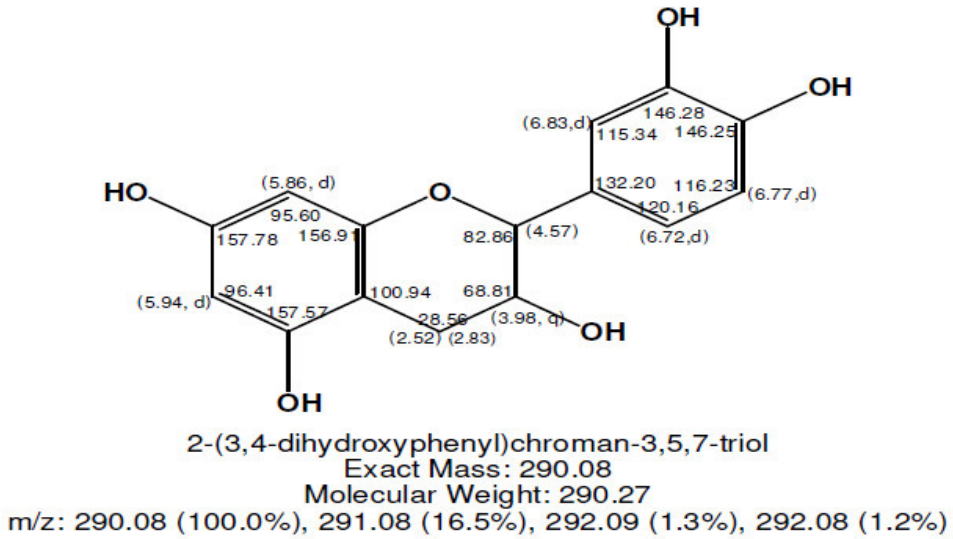

Figure 2. Structure of catechin showing ^{1}H and ^{13}C chemical shifts as elucidated using NMR, 500MHz

The ethanol and ethyl acetate extracts as well as the aqueous extract after ethyl acetate extraction and residue from ethanol extraction were tested for free radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH). All the

extracts had high activity for DPPH inhibition but moderate activity for inhibiting glucosidase *in vitro*. Catechin was identified as the major bioactive compound present in these extracts. The authors write linear relationship curve, in most cases the curves are not linear. The text could have been improved. However, this paper gives a new direction to traditional medicine study and analysis using modern analytical techniques (Apea-Bah et al. 2009, in this issue).

The widespread use of herbal remedies and healthcare preparations are described in ancient texts like the Vedas and the Bible. Plants produce bioactive molecules useful in medicines. Extensive work has been done on ethnomedicinal plants in India and the effects of plant extracts on microbes (Erdoorul, 2002). In the present study, Durga et al. (2009, in this issue) deliberate on the effect of *Acalypha indica* and *Ocimum basilicum* plant extracts on human pathogens like, *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus sp*. Ethanol extract of *A. indica* and *O. basilicum* were more effective against the pathogens than the acetone extract. Both the plant extracts have antioxidant properties, which might be due to the presence of tannins, flavonol, terpenoid or alkaloids (Oudhia, 2003). Further study can be done to identifying and isolating the active components responsible for antibacterial activity of these plants.

Casimiroa pringlei (S. Watson) Engl. (family Rutaceae, native from Mexico and Central America)is used in infusions and macerations to induce sleep and as an anxiolytic. The effect of essential oil from C. pringlei (EOCp) on an animal model is investigated in this study, which reaffirms the use as sedative and anxiolyte (Landaverdea et al. 2009, in this issue).

Guiera senegalensis (family Combrataceae, local name in Khartoum – Ghubeish) is known to have flavonoids (catechin, myricitrin, rutin and quarterin), saponins alkaloids, tannins and mucilage. Leaves are used orally for treatment of febrifuge, hyperglycemia and hypertension. Root has antileprosy properties. The plant is to treat venereal diseases like Herps Simplex Virus type 1 (HSV1). Although *G. senegalensis* has a wide range of traditional uses virtually up to now no data were available on its toxicity (Azza et al. 2009, in this issue). These data were necessary before the use of the plant in large scale for medicinal purposes. Thus in this study experiments were setup in order to study the toxicity of the plant on rats. Treatment with *G. senegalensis* resulted in endotheliotoxicity, hepatonephropathy and pancreatic hyperplasia. The plant leaf can be used safely at lower doses for its hypoglycemic properties (Azza et al. 2009, in this issue).

Lagenaria breviflora Robert (family Cucurbitaceae) used in West Africa for a wide range of gastrointestinal disorders and measles in man, for the treatment of Newcastle disease and coccidiosis in animals, as an anti-implantation agent, abortificient, miracicide and cercaricide, as well as an antibacterial agent with broad spectrum activity (Tomori et al., 2007). Phytochemical analysis showed the presence of saponins, phenolic acids and cucurbitacins (Wakimoto, 2008). Saba et al. (2009, in this issue) reported the toxicological effects of ethanolic extracts of the whole fruit of *L. breviflora* which is akin to the aqueous extract of the plant used in folk medicine in West Africa. Haematological indices (PCV, Hb concentration, MCV, MCH, MCHC values, RBC, WBC, neutrophils and lymphocytes counts) and serum electrolyte(Na+, Cl-, HCO3-, K+, Ca2+ and HPO4-) levels of Wistar rats were studied. The extract induced an increase in the mean PCV, RBC, WBC, Hb and MCV value of rats in the test groups, while MCH and MCHC decreased. The increase in the mean value of MCV, RBC and PCV coupled with decreased values of MCH and MCHC indicated increased production of reticulocytes (reticulocytosis), which suggest that the extract of the plant is capable of stimulating erythropoiesis. There was significant ($P < 0.05$) reduction of HCO3- and Ca2+, and elevation of blood urea nitrogen in the rats in the test groups. Prolonged administration of extract of *L. breviflora* elicit electrolyte imbalance but it is not haematoxic, rather, it stimulate erythropioesis and enhance immunity especially the cell-mediated immunity. The authors' elegant study shows that ethno-medicinal administration of the plant extract be done over a short period of time to avoid haemotoxicity development and renal failure (Saba et al. 2009, in this issue).

Atherosclerosis, which is one of the major causes of mortality due to cardio-vascular risks, is caused by the deposition of lipid, calcium and other substances of blood deposition in the endothelium of medium and large arteries (Nitenberg et al., 2006). Epidemiological studies resulted in an association between consumption of apples and reduced risk of some cancers, cardiovascular disease, asthma, and diabetes, lipid oxidation, and cholesterol (Boyer et al., 2004). This study was conducted to measure the acute effect of apple juice on biochemistry indices of atherosclerosis in small laboratory animal (Asgary et al. 2009, in this issue). No significant difference was found between apple juice taking groups and hypercholesterolemic diet group in TC, HDL-C, TG, LDL-C, ApoA, ApoB, SGPT, SGOT, nitrate and CRP. These results suggest that apple juice could be protective in postprandial use of on atherosclerosis due to its antioxidant effect(Asgary et al. 2009, in this issue). However, further research is needed to confirm these postulates and effects of high doses of apple juice can be of importance in future.

Emergence of multidrug resistant bacterial strains urged the investigation for alternative medicines. The present study was designed to evaluate the mode of action of the crude methanolic extract of *Hemidesmus indicus* root against *Salmonella typhimurium* both *in vivo and in vitro* (Das and Devraj 2009, in this issue). According to the ancient Indian traditional medicine system -Ayurveda, *Hemidesmus indicus* R. Br. (English: Indian Sarsaparilla, Sanskrit: Anantamul) (Family: Asclepiadaceae) is used for rheumatism, leprosy, impotency and skin infections. The root has antiulcerogenic property (Anoop and Jegadeesan, 2003) and anti-thrombotic and antioxidant activity (Mary et al., 2003). Most of the studies for antibacterial activity of natural products had dealt with the growth inhibitory effect, using simple techniques like disc diffusion, agar well diffusion or minimum inhibitory concentration determination. We had reported the antienterobacterial activity (Das and Devaraj, 2006a) and the protective effect of glycosides of H. indicus root against S.

typhimurium-induced cytotoxicity in host cells (Das and Devaraj, 2006b). The present investigation represents the effect of H. indicus root extract on S. typhimurium at its protein expression level. ME1 (methanol extract 1- insoluble fraction comprising terpenoides, steroids and fatty acids) and MHI (crude methanol extract with tannins and glycosides as the major constituents) The in vivo multiplication rate of wild *S. typhimurium* in the liver and spleen of mice was 100 fold less for MHI treated bacteria and negligible for ME1 treated *S. typhimurium*. The in vitro screening showed inhibition of type III secretory proteins (TTSPs) SPI-1 (involved in invasion and enteritis) as well as SPI-2 (involved in intracellular survival and multiplication) considerably. Differential activity of different constituents was observed to protect rat intestine as MHI had prophylactic and ME1 had therapeutic activity. It is concluded from this study that the root extract can be used as an effective remedy for *S. typhimurium* induced inflammation (Das and Devraj 2009, in this issue). Further research is needed to find out its effect against other enterobacteria and prime immunity with these microbes.

Etuk et al. (2009, in this issue) made a ethnobotanical survey of medicinal plants use in Sokoto state of Nigeria for the treatment of diarrhoeal disorders. Ten top rank plants were then selected and screened for acute toxicity, phytochemical constituents and antidiarrhoea properties. Tannin was the only phytochemical compound detected in all the screened plants and *Lannea acida* showed the highest antidiarrhoea potential.

Jahan et al. (2009, in this issue) studied *Abrus precatorius* L. (Paplionaceae) as one of the antifertility herbs used in south Asian countries (Ross, 2005).The seeds are used in birth control, as purgative, emetic, tonic, aphrodisiac, anti-ophthalmic, antiphlogistic and aphrodisiac. The chemical constituents of the seed extract are glucoside, abrusic acid, haemagglutinin, poisonous proteins, a fat splitting enzyme and abrin (Bhatt et al., 2007). Similarly its anti-motility effect has been demonstrated on washed human spermatozoa (Ratnasoorya et al., 1991). In vitro studies of isolated abrin protein from A. precatorius exhibit oxidative activity leading to DNA fragmentation in somatic cells (Narayanan et al., 2004) indicating a possibility of DNA damage induction by A. precatorius in spermatozoa along with its antifertility activity. Present investigation confirms a contraceptive or antifertility role of the seed extracts of *A. precatorius* with the possible risk of DNA damage or genotoxicity in spermatozoa and teratogenic effects (Figure. 3). It is suggested to have further investigations on the isolation of such bioactive compounds from these seeds with contraceptive activity without any genotoxic effect or DNA damaging activity in spermatozoa.

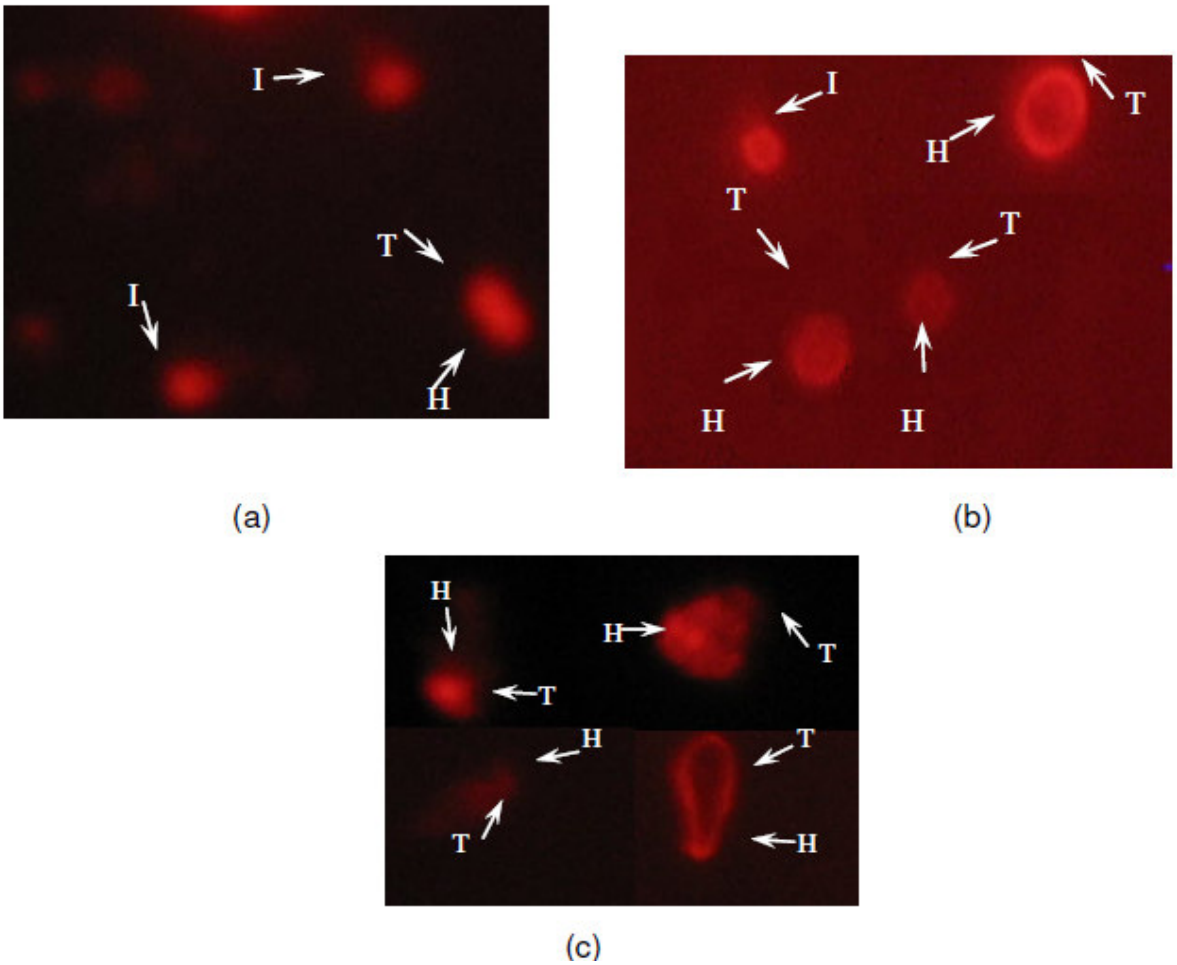

Figure 3. Fluorescent photomicrograph(x400) of adult mice sperm DNA after A. precatorius treatment using comet assay, stained with acridine orange, showing (a) control with more intact DNA sperms, (b) few comets with short tails in 20mg/kg treated and (c) more comets with long tails in 60 mg/kg treated mice. I= Intact cell, H= Head of the comet and T= Tail of the comet.

Significant attention has been paid recently to discover natural compounds safe and efficient against viral respiratory diseases. HSV-1 is widespread, enveloped and double stranded DNA agents which cause various infections in human and belongs to herpesviridae family. The virus causes recurrent infections of the nervous system located around the lips, in the eyes, in the mucous membrane of the oral cavity and genital as well (Fields, 2007a). Thus, the development of novel antiviral agents against this virus is still an important area of research. On the other hand, poliovirus is a non-enveloped virus which has single-stranded RNA. When the virus spreads to the central nervous system, it may develop paralytic poliomyelitis (Fields, 2007b). The incidence of paralytic poliomyelitis has been reduced over the last decades, especially by the systematic use of a vaccine; however, the disease is still endemic in Asia and Africa (Felipe et al., 2006). However, despite of several different *in vitro* studies, we haven't been seen any report about clinical using of natural specific compound against poliovirus (Zandi et al. 2009, in this issue). *Avicennia marina* (Forssk.) Vierh, has been traditionally used for treatment of rheumatism, small pox, ulcers and other ailments (Bandaranayake, 2002) but its antiviral activity on poliovirus and herpes simplex virus type-1 is not yet reported. In the present study, the authors evaluated *in vitro* antiviral activity of a leaf crude extract against HSV-1 and polio virus, and confirmed their efficacy in antiviral efficacy. Extract of *A. marina* showed little cytotoxicity side effects (Premanathan et al., 1999). Because of a powerful antiviral properties of the plant extracts, elucidation of the antiviral mechanism(s) of this plant extract will help in understanding the complex interactions between viruses, compounds and cells and different susceptibility of viruses(Zandi et al. 2009, in this issue).. Also, further investigation on the antiviral activity of this plant on other viruses could be interesting.

Geophyte species are often considered as a source of carbohydrates. Unlike seed storage proteins, very little is known about the reserve proteins of the underground storage organs of geophytes (Gulmaraes et al., 2001). Many kinds of alkaloids have been identified in *Colchicum* and *Merendera* plants. The major alkaloid of *Colchicum* is colchicine. The use of colchicine for treatment of gout was propounded by different researchers. Moreover, colchicine has an inhibitory effect on the growth of certain tumours in plant and animals. Colchicine acts on the mitotically active cell producing metaphasic arrest, often resulting in a doubling of the chromosome number and giving rise to polyploids. All parts of *Colchicum* species have been shown to contain colchicine, but seeds and corms contain more colchicine than other plant parts. Numerous studies have been carried out by different researchers on colchicine and other chemical constituents of *Colchicum* species (Düsen and Sümbül, 2007). In this study, Mammadov et al. (2009) examined the antioxidant and antimicrobial properties of *Colchicum balansae* Planchon (CB). The solvent extracts tubers and leaves exhibit high antioxidant activity. Ethanol extracts of CB tubers and leaves had a weak bactericidal activity. Free radicals are responsible for aging and causing various human diseases (Mammadov et al. 2009). Epidemiological studies have shown the beneficial effects of diets rich in vegetables, fruits and grain products in reducing the risk of cardiovascular disease and certain cancers (Beecher, 1999). The antioxidative activity of tuber and leaf extracts of *Colchicum* species could be exploited as an antiaging, anticancer and antistoke formulations.

Flavonoids are widely distributed in the plant kingdom and considered as natural antioxidant. In this experimental study, Zhao and Zhang (2009) report the cytoprotective effects of three flavonoids, kaempferol, quercetin and myricetin, against human hepatocytes (HL-7702 cell line) oxidative injury induced by H_2O_2 or CCl_4 were evaluated *in vitro*, with a-tocopherol as a standard antioxidant. Biochemical assays were carried out to determine the effects of the addition of three polyphenols on the hepatocytes subjected to oxidative injury, including cell viability, the content of reduced glutathione in cells, lactate dehydrogenase leakage into culture medium and the formation of malondialdehyde in hepatocytes. The intensity of the potential cytoprotective effect of three flavonoids evaluated qualitatively (Figure 4) was in the order of quercetin>myricetin>kaempferol, which did not show a clear structure (Figure 5)-activity relationship between the numbers of hydroxyl group in ring B of the compounds and their cytoprotective effect (Zhao and Zhang 2009, in this issue).

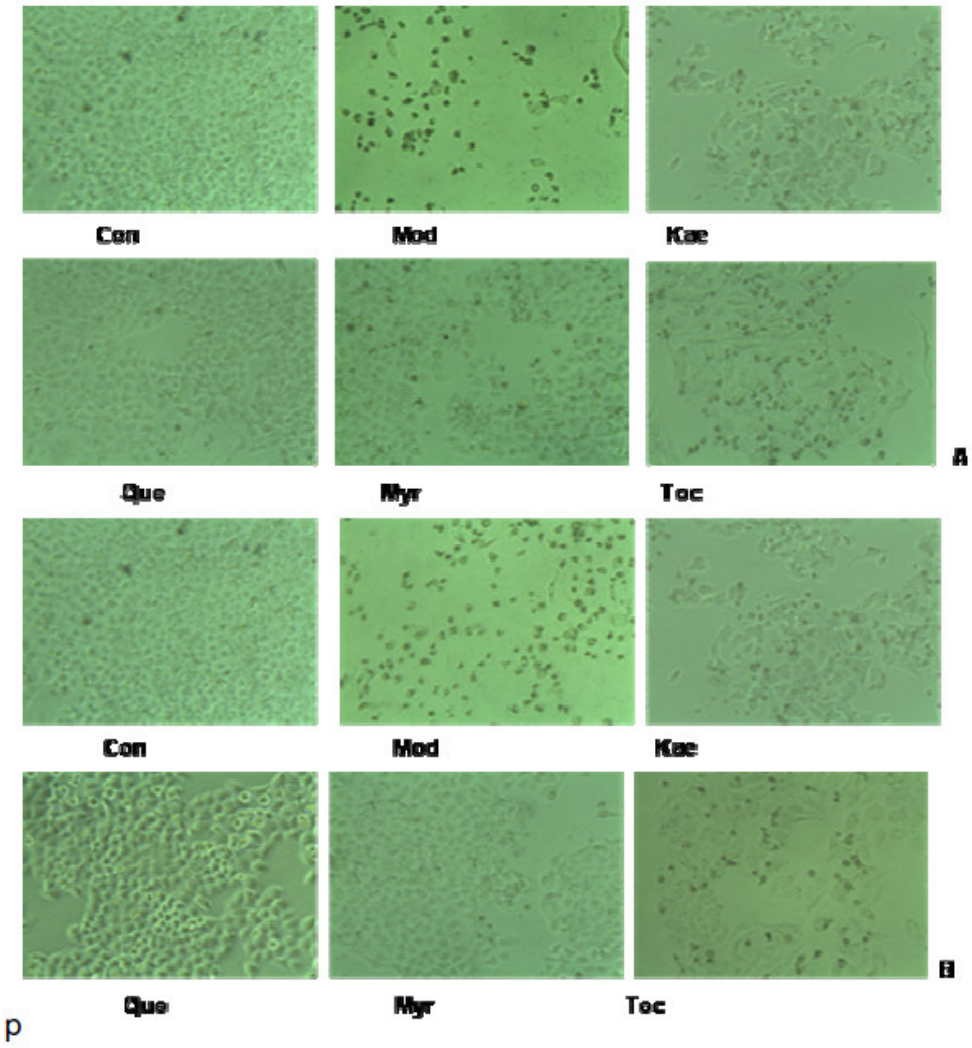

Figure 4. Morphological observations of oxidative injury of hepatocytes induced by addition of H_2O_2 (A) or CCl_2 (B) *in vitro*.
Hepatocytes were incubated with three flavonoids and α-tocopherol at 60 μmol/L for 30 min, the treated with H_2O_2 for 1.5 h or CCL_2 for 6h. Con: control group. Mod; model group, Kae; kaempterol group, Que; quercetin group, Toc; α-tocopherol group. Hepatocytes were observed at x100 magnification.

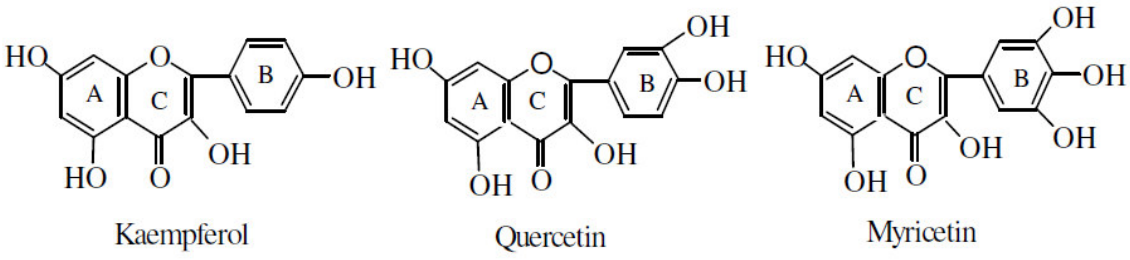

Figure 5. The chemical structures of kaempterol, quercetin and myricetin

From the above studies it is imminent that most of plant products, derivatives and their active ingredients are either antioxidants or modulate anitoxidative responses in model animals to induce disease or stress resistance. So a detailed survey of antioxidants in plants and their therapeutic potential may be useful for inventories medicinal plants for pharmaceutical use.

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