DISCUSSION

Researchers are looking to various alternative systems of medicine for providing some leads for the treatment of some chronic diseases. Herbal drugs, which are claimed to be safe while being equally effective in comparison to allopathic drugs, are receiving particular attention in this regard. However, these herbal drugs are often marketed with exaggerated claims or in some cases are credited with innumerable pharmacological activities, which are not mentioned in the text of various traditional systems of medicine. If we compare the strengths and weaknesses of herbal medicines with those of modern medicines, we find that the former have a strong traditional or conceptual base and potential to be used as drugs in terms of safety and effectiveness, but lack an experimental base and therefore have second-class status in comparison to modern medicines which have a very strong experimental basis for use but may be potentially toxic. Thus with the view to getting a new class of drugs, researchers are increasingly blending the traditional knowledge with modern experimental methodology for testing the efficacy and safety of herbal drugs.

Drug discovery from medicinal plants has evolved to include numerous fields of inquiry and various methods of analysis. The process typically begins with botanists, ethno botanists, ethno pharmacologists or plant ecologists who collect and identify the plants of interest. Collection may involve species with known biological activity for which active compound(s) have not been isolated (e.g., traditionally used herbal remedies) or may involve taxa collected randomly for a large screening program. It is necessary to respect the intellectual property rights of a given country from where the plant(s) of interest are collected. Phytochemists prepare extracts from the plant materials, subject these extracts to biological screening in pharmacologically relevant assays, and commence the process of isolation and characterization of the active compound(s) through bioassay-guided fractionation. Molecular biology has become essential to medicinal plant drug discovery through the determination and implementation of appropriate screening assays directed towards physiologically relevant molecular targets. Pharmacognosy encapsulates all these fields into a distinct interdisciplinary science. Herbal products are sold either as raw
plants or extracts of portions of the plant. Both the raw herb and the extract contain complicated mixtures of organic chemicals, which may include fatty acids, sterols, alkaloids, flavonoids, glycosides, saponins, tannins and terpenes.

The secondary metabolites produced by plants can have therapeutic action which can be refined to produce drugs. The word drug itself comes from the Swedish word "drug", which means “dried plant”. Some examples are inulin from the roots of dahlias, quinine from the bark of cinchona, morphine and codeine from poppy, and digoxin from foxglove. The active ingredient in willow bark is salacin or salicylic acid and the discovery of salicylic acid led to the development of 'aspirin', also known as 'acetylsalicylic acid'.

A significant part of drug discovery in the last forty years has been focused on agents to prevent or treat cancer. This is not surprising because in most developed countries and to an increasing extent in developing countries, cancer is amongst the three most common causes of death and morbidity. Treatment for cancer may involve surgery, radiotherapy and chemotherapy; and often a combination of two or all three are employed (Dasgupta et al., 2004). Cancer is the second leading cause of death in the United States, surpassed only by cardiovascular disease (Noguera et al., 2004). Although these figures are disquieting, some progress has been made in cancer diagnosis and treatment as evident from the high incidence of breast, prostate, testicular and uterine cancer as compared with their relatively lower mortality (Benencia et al., 2000; Noguera et al., 2004; Kuete et al., 2008).

Drug discovery from medicinal plants has played an important role in the treatment of cancer and indeed most new clinical applications of plant secondary metabolites and their derivatives over the last half century have been towards combating cancer (Chakraborty et al., 2002; Owoyele et al., 2005; Ratnasooriya et al., 2005). Of all the available anti-cancer drugs developed between 1940 and 2002, 40% were natural products per se or natural product derived, with another 8% considered as natural product mimic (Chakraborty et al., 2002). Natural compounds from flowering plants play a significant role in cancer chemotherapy. Anti-cancer drugs in wide clinical use include vincristine
and vincristine from *Catharanthus roseus*, paclitaxel (Taxol) and taxotere from species of yew (Taxus), etoposide derived from lignans of *Podophyllum* spp and its analogues such as topotecan, and camptothecin from *Camptotheca acuminata*. All of these are fundamentally cytotoxic and act principally by inhibiting cell proliferation but by different mechanisms. In fact, some natural products have been found to act by novel mechanisms and so have enabled novel targets to be developed by screening. This may be exemplified by the discovery that paclitaxel inhibited mitosis by stabilizing microtubules and so preventing their depolymerization back to tubulin in contrast to many other anti-cancer agents which inhibit the formation of microtubules in the first place (Seki *et al*., 2010).

In spite of these successes there is still much activity directed to find novel anti-cancer agents. The traditional cytotoxic approach is associated with severe and unpleasant side effects in clinical usage. Hence a "cocktail" of lower doses of such compounds is now often given, rather than a large and therefore more toxic dose of a single compound. It is relatively easy to screen extracts and compounds for cytotoxic effects in high through out automated screening procedures that are used in industry and by research organizations such as the National Cancer Institute in USA, which employs 60 different cancer cell lines.

So, it could be concluded that in spite of all the challenges, plants and plant derived products will continue to be an important and invaluable source for the discovery of new drugs. It is expected that with research organizations, pharmaceutical companies and academic institutions exploring plant materials for medicinal properties, a number of new and safe medicines may emerge from herbal sources to combat some of the diseases like cancer for which no suitable medicine exists.

One of the goals of cancer chemotherapy is to explore and develop new molecules and/or therapies, which can selectively induce apoptosis in cancer cells (Denicourt *et al*., 2004). The study reports, for the first time, the anti-cancer activity of methanolic extract of aerial parts of *C. acutangulus* in some cancer cell lines. Accordingly, the first phase of
the study deals with bioactivity guided fractionation and identification of corchorusin-D which was the active ingredient responsible for this activity. The study also provides a novel insight into the mechanisms involved in COR-D induced early events leading to the activation of signaling cascades and culminating in apoptotic cell death in human histolytic lymphoma (U937), promyelocytic leukemia (HL-60), chronic myeloid leukemia (K562) and murine melanoma (B16F10) cell lines. The results demonstrated that the compound inhibited proliferation of U937 and HL-60 cells in a concentration-dependent manner. The study reveals that COR-D induced mitochondrial dysfunction in U937 and HL-60 cell lines, and triggered the mitochondrial intrinsic pathway by release of AIF from mitochondria and translocation of Bax from cytosol to mitochondria. This facilitated caspase 9 activation and up regulation of downstream pathways leading to caspase 3 activation and PARP cleavage. The involvement of extrinsic pathway in apoptosis by increase in activation of caspase 8 confirmed this contention.

Further, the efficacy of the compound was tested on K562 (chronic myeloid leukemia) cell line, which is formed through a reciprocal translocation between chromosomes 9 and 22, giving rise to the constitutively active cytoplasmic protein tyrosine kinase P210 BCR/ABL and initiating signaling through multiple pathways (Heisterkamp et al., 1985). Significant annexin V-FITC positivity, accumulation of cells at sub-G0 phase, and typical morphological changes indicated that COR-D induced apoptosis in K562 cells. The induced apoptosis followed the mitochondria-dependent intrinsic pathway by decreasing mitochondrial membrane potential, and Bcl-2/Bax ratio and releasing cytochrome c from mitochondria to cytosol, facilitating caspase 9 activation. It also up-regulated the downstream pathways leading to caspase 3 activation and PARP cleavage. There was no significant change in pro-caspase 8 and Bid expression: as Bid a Bcl-2 family protein, is the substrate of activated caspase 8 whose activation is regulated by the death receptor, mainly through Fas/FasL interaction (Zimmermann et al., 2001) and K562 is Fas/FasL null. However, COR-D induced caspase 10 activation in K562 cells, which indicated that the death receptor pathway is activated by TNF receptor rather than Fas/FasL. The association between TNF-R1 and TRADD was maximum at low concentrations of COR-D; at higher concentrations, cells underwent apoptosis by COR-D induced destabilization.
of the complex. This proves that the death receptor pathway was also activated. On investigating the role of COR-D on AKT/PKB pathway, it was found that the compound activated the upstream kinase PDK1, which in turn suppressed AKT at Ser-473 and Thr-450 sites. Thus COR-D inhibited the AKT/PKB pathway to enhance apoptosis by activating mitochondrial pathway. Investigations on the role of the MAP kinase family members in regulating COR-D induced apoptosis showed that ERK and the upstream kinase MEK were up-regulated. Activation of the ERK pathway can also occur in the absence of Ras activation (Burgering et al., 1993). It was noticed that COR-D could neither activate Ras nor suppress ERK. Instead cell death was enhanced after inhibiting MEK, which suggested MEK dependent ERK up-regulation in COR-D induced cell death. JNK and p38, the two kinases associated with different cellular processes including cell growth, differentiation, transformation and apoptosis (Johnson and Lapadat 2002; Werlen et al., 2003), were activated in response to COR-D treatment. But cell death was enhanced when JNK and p38 were pre-inhibited with their specific inhibitors. This implied that the activation of p38 and JNK was associated with cell survival or cellular protection. In all, COR-D could induce apoptosis in K562 cells by activating intrinsic and extrinsic pathways and suppressing the AKT/PKB pathway. However, the MAPK family members ERK, p38 and JNK were not involved in the apoptotic response. Thus COR-D in combination with MAP kinase inhibitors is capable of enhancing apoptosis in K562 cells.

After observing the activity of COR-D on leukemic cell lines it was necessary for us to know whether COR-D has any effect on other cancers. It has also been reported that saikosaponin b2 inhibited the proliferation of melanoma cells (Zonga et al., 1996; Zong et al., 1998; Fujioka et al., 2003). Based on the structural similarity between saikosaponin D and COR-D, the pharmaceutical activity of COR-D has been investigated. The study reports, the apoptotic effect of COR-D and the mechanisms involved in COR-D induced activation of signaling cascades culminating in apoptotic cell death in B16F10 melanoma cell line and in vivo mice model. The results demonstrated that COR-D inhibited cell proliferation of B16F10 cells in a concentration-dependent manner. Further investigations showed that cultured B16F10 cells treated with COR-D exhibited
morphological features of apoptosis and increase in PI positivity, which supports the contention that cell death after COR-D treatment occurs via apoptotic signaling pathway. The product altered the Bax/Bcl-2 ratio and triggered the mitochondrial pathway of apoptosis. The activation of the caspase cascade indicated that the promotion of apoptosis in response to death inducing signals originated from cell surface receptors, mitochondria or endoplasmic reticulum. The study also revealed that the compound induced elevation of some caspases, including caspase 3 and 9. Activation of initiator caspases such as caspase 9 in response to the pro-apoptotic signals activates caspase 3, the major effector caspase (Patel et al., 1996), and this plays the pivotal role in the initiation of apoptosis (Nicholson et al., 1995; Salvesen and Dixit, 1999). It was also found that COR-D inhibited the B16F10 tumor growth and increased the survival rate of mice. The reduction in tumor growth is well correlated with the decrease in microvascular density and mitotic index of the tumor cells. Most importantly, animals did not show any change in visual symptoms like hair loss, weight loss, diarrhea and movement with doses of 25 and 50 mg/kg body weight. In conclusion, this study reveals that COR-D-induced mitochondrial dysfunction is responsible for the induction of apoptotic cell death both *in vitro* and *in vivo*. 
CONCLUSION

The study was taken up in order to test the efficacy of a natural product (COR-D) against leukemia and melanoma. The compound was identified as a constituent of *C. acutangulus* with structural similarity with saikosaponins, many of which possess profound anti-leukemic and anti-melanoma activity. COR-D was tested in U937, HL-60, K562 and B16F10 cell lines. It appears to act in mitochondrial dependent intrinsic pathway in U937, HL-60 and B16F10 cells. COR-D appears to act through the activation of intrinsic and extrinsic pathway of apoptosis and suppression of AKT/PKB pathway. It could not suppressed the MAP kinase pathway but the enhancement of apoptosis was noticed when ERK1/2, p38 and JNK1/2 were preinhibited with specific inhibitors, suggests that COR-D can enhance apoptosis in K562 cells in combination with MAP kinase inhibitors. Thus the finding point to the possibility of developing COR-D and its analogous as anti-leukemia and anti-melanoma activity.
REFERENCES


