ABSTRACT

We have extracted aerial parts of the Tragia involucrata successively with hexane, toluene, dichloromethane, acetone, methanol and water. The crude extracts were subjected to phyto-chemical analysis. Hexane extract showed the presence of sterol and terpenoid while the acetone and methanol extracts were positive for the presence of flavonoids.

TLC analysis of the hexane extract after defatting showed two spots in the solvents; cyclohexane: ethyl acetate (4:1). It was subjected to adsorption column chromatography and eluted in different solvents with various combinations. Column chromatography resulted in the isolation of terpenoid and sterol with Rf values of 0.76 and 0.47 respectively which was confirmed by the physical analysis and chemical tests. The sterol and terpenoids were dissolved in methanol and crystallized at low temperature. The crystals of terpenoid were further analyzed by UV, IR, NMR, LC–MS and CHN analysis to elucidate the structure of terpenoid.

Ethyl acetate extract was also subjected to TLC with different solvent combinations. It was subjected to column chromatography to separate the constituents but in vain. Hence, it was separated by preparative TLC. The isolated flavonoid was confirmed by physical and chemical analysis. The structure of purified flavonoid was elucidated by UV, IR, NMR, LC–MS and CHN analysis. The structure of the compound is, 5-(3-hydroxy-propyl)-benzene-1, 2, 3-triol (HPBT).

Hexane, toluene, dichloromethane, acetone and methanol extracts were studied for their in vitro effects on cancer cell lines such as KB, MCF7, Vero and BHK. The IC50 values ranged from 0.88mg-177mg/ml. Methanol extract showed a significant cytotoxicity against all the cell lines with IC50 values of 1.76± 0.02, 2.58 ± 0.54, 0.88 ± 0.01, 0.127 ± 0.02mg/ml for KB, MCF7, Vero and BHK cell lines.
respectively. Hexane extract also showed a significant anti-proliferative activity when compared to other extracts with IC_{50} values ranging from 1.34±0.03 to 5.7±0.39mg/ml.

The anticancer efficacy against Ehrlich's Ascitic Carcinoma has provided evidence of major anticancer activity for HPBT and terpenoid. Single or multiple intraperitoneal (i.p.,) dose of drugs provided high level activity against the subcutaneous (s.c.,) grafted EAC, significantly increase in life span. The ILS% for HPBT was 18.18, 25 and 104.54% for the single dose and 4.54, 52.27 and 75% for the multiple doses at the dosage of 60, 75, and 90mg/kg body weight respectively. Even the Terpenoid showed significant antitumor activity against EAC solid tumor model at the dose of 50, 100 and 150 mg/kg body weight. Terpenoid was non-toxic even when the concentration was increased up to 2000mg/kg body weight.

HPBT exhibited significant antioxidant activity in ABTS and DPPH assays and the IC_{50} values found to be 0.85 ± 0.012 and 6.46 ± 0.371µg/ml respectively. In addition, in vivo antioxidant activity of the HPBT was carried out. The administration of HPBT at 50mg/kg body weight for 7 days or a single dose caused a significant increase in the level of total antioxidant, Nitric Oxide and SOD level in the Serum. The antioxidant activity showed reciprocal dose dependence. At 50mg/kg body weight the activity found to be highest while there was a decline in the antioxidant capacity as the concentration was increased to 90mg/kg.

Evaluation of new chemical entities for genotoxicity is an important part of the safety analysis of new drug substances. Thus the one of the objective of the present study was to determine the genotoxic potential of the HPBT in terms of induction of chromosome aberrations (CA), micronuclei test (MN) and sperm abnormality assay.
In CA assay the drug induced statistically significant individual type of aberrations. This indicates the strong genotoxic potency of these compounds. A statistically significant MN induction in PCEs and NCEs in different doses and time intervals confirms the clastogenic potency of this chemical. The induction of MN by this drug may be due to chromosomal breaks or due to the lagging of whole chromosome, while subsequently was not included in the main nuclei.

HPBT and terpenoid appeared to have good anti-inflammatory and analgesic activity. Terpenoid showed a significant anti-fertility, anti-inflammatory and analgesic activity; whereas the HPBT was not active as anti-fertility agent but, it showed a considerable anti-inflammatory and analgesic activity. A dose dependant anti-implantation response was observed for Terpenoid. At the dose of 100 and 200mg/kg body weight showed 63.71 and 81.33% (p<0.001 compared to control) anti-implantation potency respectively. Administration of these extracts caused a significant increase in uterine weight in immature rats (versus control, p< 0.001). The uterotrophic potency was about 95% and 88% for HPBT at the dose of 50 and 100mg/kg and 59 and 53% for terpenoid at the dose of 100 and 200mg/kg body weight respectively of that of ethynil estradiol. The HPBT and terpenoid of T.involucrata provided remarkable anti-inflammatory activity ranging between 41.25-66.54% (p<0.02) and 20.20 – 53.19% (p<0.02) respectively HPBT and Terpenoid significantly and dose dependently reduced the intensity of acetic acid induced abdominal constriction in mice. The effect of higher dose of Terpenoid was comparable to that of aspirin (200mg/kg)
Conclusison:

We have isolated a terpenoid and a flavonoid from T. involucrata and shown in the present thesis that they exert growth inhibitory effect on human cancer cells and hence will make a novel natural chemical source for anticancer drugs. Efficacy of these drugs against the in vivo anti-tumor assay, antioxidant activity as well as the anti-inflammatory and analgesic activity provides the strong evidence about their potency as natural cancer chemotherapeutic agents. Flavonoids showed the genotoxic effects comparable to anticancer drugs. In any case, terpenoids and flavonoids with novel structure are hopeful candidates for anticancer drugs.