5. DISCUSSION

Medicinal plants have played an important role in maintaining human health and improving the quality of human life since many years. The use of natural products for therapeutic purposes is in practice from ancient times.

The world health organization has estimated that 80% of the earth inhabitants relied on traditional medicine for their primary health care needs. Herbs have been used as food and medicine from many countries to cure the diseases. The traditional medicines are used to cure all kind of diseases especially they were believed to possess anti-tumor or immune stimulating properties. Several herbs and plants have reported to have antifertility activity both in male and female. Most medicinal plants are considered as potential source of antifertility compounds. The antifertility activity is imported in medicinal plants by the presence of secondary metabolites.

Phytochemicals analysis of plants extracts indicates the presence of primary and secondary metabolitessuch as phenols, alkaloids, glycosides, flavonoids, and tannins which possess antifertility activity.

PHYTOCHEMICALS

The beneficial medicinal effects of plant materials typically result from the combinations of secondary metabolites present in the plant, such as alkaloids, flavonoids, steroids, tannins and phenolicterpenes, volatile oils which are synthesized and deposited in specific parts or in all parts of the plant. The plant secondary products may exert their action by resembling endogenous metabolites, ligands, hormones, signal transduction
molecules or neurotransmitters and thus have beneficial medicinal effects on humans due to similarities in their potential sites. Therefore, random screening of plants for bioactive chemicals is as important as, the screening of ethno botanically targeted species.

Phytochemical screening of *Cassia auriculata* L extract has revealed the presence of alkaloids, flavonoids, Phenolic, steroids and saponins in petroleum ether, chloroform and ethanol extracts as reported by Manjush R W *et al.*, (2010).

In present study, the areal part of *P. oleracea* L has been subjected to extract crude drug in 3 different solvents such as petroleum ether, chloroform and ethanol by Soxhlet extraction method, the crude drug so obtained was tested for phytochemical studies which reveals the presence of phyto-constituents such as alkaloids, phenols, glycosides, flavonoids, tannins, gums and mucilayes and quantitative estimation study indicates the presence of carbohydrates, proteins, amino acids, reducing sugars, total phenols and taninins etc. in all the above 3 different organic solvent extracts. There are some other several secondary metabolites present in plant extracts. The quantity of primary and secondary metabolites present in the plant sample is also important for their biological activity is also been estimated.

The studies of Baskarn *et al.*, (20011) on *Murrayakoeni* indicates the presence of alkaloids, proteins and amino acids, phenolic, saponins, flavonoids, terpinoids present in petroleum ether, chloroform extract and ethanol extract.
Similar studies of Chinnapan, (2011) also reveals that phytochemical analysis of *Andrographisalata* indicates the presence of saponins, flavonoids, triterpenoids, glycosides, gums and mucilages, tannins, phenolic compounds and steroids, in petroleum ether, chloroform and ethanol extracts.

**PHARMACOGNOSY**

In recent years, the pharmacognostic studies have been sustained its claim to rank as science, However the collection of herbs for medicinal use. The detailed description about plant and its habita, soil suitability for their growth, the season of collection of plant, the duration of efficacy of the metabolite and their storage are mentioned in Ayurveda and medicinal lexographs. Ayurveda literature from the time of Charaka and Shushrutha shows a picture of systemic development. This made the path of knowledge behind the bounderies of India such as Greek, Egypt and Rome. There are two main factors important for standardization or evaluation of natural products one is raw material standardization and the other is process standardization. Recently WHO encouraged the use of medicinal plants in developed countries; they accept Indian and Chinese system of medicine. As there is increase in demand for raw drugs, to fulfill the demand, manufacturers face some problem regarding supply of raw material. To meet these demand suppliers collect the drugs irrespective of proper age, season, part, etc. This leads to adulteration and substitution of herbal drugs.

Raw material standardization can be done by identification of plant and evaluations like organoleptic properties. It includes microscopic study, color, odor, taste, moisture
content, ash content, and assay of minerals. The ash value is useful in determining authenticity and purity of the drugs. Fluorescent study is also conducted to determine the fluorescence and color of the extracts. It is therefore important to standardize the plant material, which can help to differentiate the genuine drug from adulterant material and if we standardize the drugs, it may be useful to check the adulteration of crude drug in industrial or research field.

In the present study the results of organoleptic properties like, color, odor, taste, moisture content, ash content and fluorescent study can be compared to the study conducted by D. C. Modi et al., (2010).

ANTIMICROBIAL ACTIVITY

Nowadays multiple drug resistance by the microbes is developed due to the indiscriminate use of drugs commonly used in the treatment of infectious disease treatment. Unfortunately, bacteria have genetic ability to transmit and acquire resistance to drugs and chemicals (Nascimento et al., 2000). The extra chromosomal genes associated with plasmids were found to be responsible for these antibacterial resistance phenotypes that may impart resistance to entire bacterial class (Motamedi et al., 2009).

Phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanisms against predation by microorganisms. In our study the three solvent extraction viz., petroleum ether, chloroform and ethanol extract of plant *P. oleracea* *L* have shown
antibacterial activity against both the gram positive and gram negative bacteria which indicates the presence of broad spectrum of antibiotic compounds in the plant. This property will be of immense advantage in fighting the menace of antibiotic refractive pathogens that are more prevalent in recent time. Different solvents have been reported to have the capacity to extract different phyto-constituents depending on their solubility or polarity and property of the solvent.

Tessefaye and Chalachew (2012) have reported the effect of *Dichrocephalinteri folia* (L.f) where the petroleum ether, chloroform and ethanol extracts have antimicrobial activity on both gram positive and gram negative bacteria.

Therapeutic value of medicinal plants and bioactivity of extract lies in the various chemicals present in it, for instance, plant rich in tannins have antimicrobial potential due to their basic character that allows them to react with proteins to form stable water soluble compounds thereby killing the bacteria by directly damaging its cell membrane (Mohamed Sham Shihabudeen *et al.*, 2010). Flavonoids are the major group of phenolic compounds reported to have antimicrobial activity (Maria Lysete *et al.*, 2009). The extracts of seeds of *Vitexagnus-castuswas* reported to possess antimicrobial activity which is associated with its alkaloids, saponins, taninns, flavonoids and glycosides contents (Arokiyaraj *et al.*, 2009). The antimicrobial activity of aerial part extracts of *P. oleracea L* as recorded in the present study may therefore be attributed to the presence of above phytoconstituents.
The flavonoids isolated from ethanol extract of *P. oleracea* L has subjected to antimicrobial studies by agar diffusion and broth assay methods. Based on the results, obtained (Graph 1 and 2) it can be ascerted that the flavonoid of *P. oleracea* L has significant activity on *S. typhimurium*, and *P. mirabilis* at 40 mg/mL of concentration when compared to control group against all the tested strains. This may be due to the chemical nature, cell membrane permeability and other factors of isolated flavonoid. According to the study of Pongsak and Parichat (2010) on antibacterial activity of flavonoids extracted from leaves of *Psidium guajava* on both gram positive and gram negative bacterial strains it exhibited that this property may be due to the presence of flavonoid quercitine, morin-3-O-arbinoids.

Flavonoids are most effective antimicrobial compound due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall, more lipophilic and they may also disrupt microbial membrane (Tsuchiya *et al.*, 1996).

In general, composition of the inhibition zones diameter showed that the flavonoids are more effective against both gram positive and gram negative bacterial strains. The difference in the activity of flavonoid may be due to several possible reason such as permeability barrier provided by the presence of cell wall with multi-layer structure in gram negative bacteria or the membrane accumulation mechanism or presence of enzymes in periplasmic space which are able to break down foreign molecules introduced from outside (Parekh *et al.*, 2007).
In our study the flavonoid of *P. oleracea* L has shown a very less activity on the bacterial strains like *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Enterobacter aerogenes* where as its activity is significant on *S. typhimurium*, and *P. mirabilis*. The minimum inhibition concentration of compound of *P. oleracea* L at 4 mg/mL, has antimicrobial activity. Based on above result it can be concluded that flavonoid of *P. oleracea* L has significant bactericidal effect on *S. typhimurium*, and *P. mirabilis* and non-significant bactericidal activity against *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Enterobacter aerogenes*.

**ANTIFERTILITY ACTIVITY OF VARIOUS EXTRACTS OF *P. OLERACEA* L IN FEMALE ALBINO RATS**

Many plant preparations and medicaments used by ancient physicians are reported to have fertility regulating properties in ancient Indian literature (Kirtikar and Basu, 1935; Nadkarni and Nadkarni, 1954; Chopra *et al.*, 1956; Evans and Huezo, 1990; Anonymous, 1996).

The use of plants as emmenagogues, abortifacient and as local contraceptives was well known to ancient physicians of India. However, the precise nature of their use or the preparation of materials out of plants used for the purpose is not clearly known. In recent times, sporadic attempts have been made by investigators to develop contraceptive agents from the alleged antifertility plants available in their locality. Studies on fertility regulating plants have been given priority by ICMR for the last 30 years but no significant lead had been obtained so far. A systematic integrated approach based on authenticated plant and proper animal models was initiated at Central Drug Research Institute (CDRI) and Council.
for Scientific and Industrial Research (CSIR) laboratories since last 30 years (Kamboj, 1988). Many workers have compiled the information related to Botany, Chemistry and Pharmacology of plants, plant parts and their products which have the antifertility property.

The plants that are having antifertility properties may be classified according to their activity profile as done in the present study.

1) Anti-ovulatory
2) Anti-implantation
3) Abortifecient
4) Estrogenic/anti-estrogenic
5) Hormonal profile

In the present experiments extracts of *P. oleracea* *L*, which are investigated for antifertility activity, have shown promising results in female albino rats. Out of three extracts viz., petroleum ether, chloroform and ethanol studied for antifertility properties, ethanol extract is the most effective and possesses prominent antifertility activities.

**Anti-ovulatory activity**

The estrous cycle of mammals reflects the changes in the ovary, uterus, adrenal and vagina. The cyclic changes that occur in these organs are under the synergistic influence of many hormones and factors. Any abnormality or dysfunction of these organs or their synchronization directly affects the reproductive phenomenon in the particular individual.
In our study it is demonstrated that, administration of petroleum ether, chloroform and ethanol extracts of *P. oleracea* L at the dose level of 250 mg/kg of body weight for 15 days to adult female albino rats has shown non-significant (*p* < 0.01) reduction in the number of ova present in the ovary, 10.00±0.32, 10.00±0.32 and 05.00±0.02 respectively, but at the dose level of 500 mg/kg of body weight treatment of petroleum ether, chloroform and ethanol extracts has shown significant (*p* < 0.01) reduction in the number of ova present in the ovary when compared to control group 08.00±0.62, 06.00±0.01 and 03.00±0.01 respectively, several researcher have studied on the antiovalation effect of plant extract. The antifertility property of Neem leaves; seeds, bark, and oil have been studied by some investigator in other parts of the world (Updhyay *et al.*, 1990; Shakti *et al.*, 1990; Riar *et al.*, 1991; Kasturi *et al.*, 2002).

**Anti-implantation activity**

The implantation and maintenance of early pregnancy is critical and complex phenomenon in mammals. Many methods and agents have been developed to inhibit the process by impairment of the phenomenon, either at the tissue level either naturally or through the agents involved.

The anti-implantation effect of plant extracts may be due to the disturbance of endocrine-endometrial synchrony which is dependent on estrogen and progesterone balance. Factors other than the hormones such as histamine, prostaglandins, proteolytic enzymes, alkaline, phosphatase, interleukins, and Leukemia inhibitory factors, which are important for implantation, may also be affected by the extracts (Endalk *et al.*, 2005).
In the present study the number of implantation sites are non-significantly ($p < 0.01$) reduced $10.67\pm0.3261$, $6.00\pm0.0039$ and $8.33\pm0.02$ due to the treatment of petroleum ether, chloroform and ethanol extracts of *P. oleracea* L respectively at the dose level of 250 mg/ kg of body weight for 1st to 7th day to the pregnant albino rats, but the high dose level 500mg/kg of body weight treatment of petroleum ether, chloroform and ethanol extracts have significantly ($p<0.01$) reduced respectively in the number of implantation sites when compared to control group $7.67\pm0.05$, $5.66\pm0.01$ and $6.66\pm0.04$.

According to the study conducted by Geremew *et al.*, (2005) 37 % of anti-implantation was exhibited by the treatment of ethanol extract of *Leonotis cymifolia* in albino rats and also the studies of Ghosh K and Bhattachary T K (2004) shows that about 60% of anti-implantation activity was observed due to the treatment fatty acids at 110 mg/kg of b w in albino rats, these investigations supports our present data. The loss of implantation caused by the fatty acids may be due to autizygotic, blastocytotoxic activity.

In the present investigation the failure of implantation process may be due to the influence of the extracts in one/many of the activities essential for implantation process. The following processes are needed for implantation phenomenon as studied by many researchers. (Maitre *et al*, 1975; Paria *et al*, 1993; Peyron *et al*, 1993; Cross *et al*, 1994; Tafuri *et al*, 1995; Guyton and Hall 1996; Ghosh *et al*, 1997; Lim *et al*, 1997; Munn *et al*, 1998; Paria *et al*, 1998; Paria *et al*, 2000; Baired, 2000; Norwitz *et al*, 2001).

- Fertilization of ovum by the sperm in the fallopian tube.
- Transport of zygote to the uterine cavity through the fallopian tube for the development.
Development of zygote in forms the blastocyst, which is responsible for implantation in uterine endometrium.

Activation of blastocyst by inhibiting the class of estradiol metabolites called as catechol estrogens either by antagonizing them or by inhibiting their biosynthesis.

Formation of corpus luteum in ovary after ovulation, which is the source for the synthesis of progesterone responsible for maintenance of pregnancy at all stages.

Secretion of estrogen and progesterone, which are responsible for uterine proliferation and uterine receptivity.

Process of apposition by inhibiting regulation factors such as leukemia inhibitory factor (LIF), prostaglandins especially prostaglandin F, cyclooxygenase enzyme (COX), interleukin (IF) etc.

Biosynthesis of COX enzymes and their action, which in turn responsible for the biosynthesis of prostaglandins.

Production of adhesion molecules and proteinase enzymes responsible for the invasion of blastocyst into uterine endometrium.

Production of growth factors and hormones such as prolactin, relaxin, leukemia inhibitory factor (LIF), etc. from the decidua under the influence of progesterone.

Synthesis, secretion and action of glucocorticoids essential for the maintenance of early pregnancy.

Placental growth and differentiation.

In the present investigation inhibition of implantation sites on 10th day of pregnancy in the rats after receiving different solvent extracts of *P. oleracea* *L* indicates
the possibility of interference of active constituents of extract in any one or some of the above processes. Further action of crude extract might have imbalanced the biosynthesis of hormones such as progesterone, estrogens and glucocorticoids by blocking the enzymes necessary for implantation.

In the present study, as the phytochemicals of ethanol extract may be caused the imbalance in steroidal hormones might be the reason for anti-implantation activity. The ethanol extract has shown more anti-implantation activity in the present study may also due to autizygotic, blastocytotoxic or anti-implantation activity as described by Hafez et al., (1970). Withdrawal of these treatments from adult rats has resulted in normal reproductive activities.

Abortifecient activity

The apparent neutralization of LH during day 7-11 of pregnancy in rat results in the termination of gestation by fetal resorption (Rothchild et al., 1974). The hypothalamus has threshold requirement for estrogen to cause a massive release of LH by the pituitary gland. This surge of LH is the trigger which initiates the rupture of the follicle (ovulation) (Bullock, et al., 1995). Initiation of a rise in the progesterone synthesis and pituitary LH release coincides with an increased follicular growth and hypertrophy of corpora lutea between day 9-12 of pregnancy in rats and hamsters (Greenwald, 1973; Rothchild et al., 1974). Therefore, it is evident that pituitary LH is essential for the maintenance of corpora lutea in the functional state as to produce progesterone sufficient to maintain the pregnancy during the early half of pregnancy. Progesterone a pregnancy hormone secreted by corpora lutea is sustained by Prolactin with FSH through day 7 and thereafter placenta will take over the function (Morishige and rothchild, 1974).
The level of estrogen secretion during pregnancy is comparatively lower compared to progesterone, as the former is in the range of nanogram and later is in microgram thought pregnancy except near term (Eto et al., 1962; Hashimoto et al., 1968). Throughout pregnancy the secretion rate of estrogen is low with periodic small peaks, which is considered to be the persistence of cyclist during pregnancy (Schwartz and Telley, 1968; Yoshinaga et al., 1969). Thus progesterone is the main hormone to maintain pregnancy, the synergistic action of estrogen secreted during gestation is also necessary.

In this present investigation administration of petroleum ether, chloroform and ethanol extracts of *P. oleracea* L at the dose level of 250 mg/ kg of body weight for 7th to 14th day to the pregnant albino rats has showed non-significant (p < 0.01) 4.00±0.21, 8.00±0.032 and 12.33±0.97 abortifacient activity respectively, but the treatment of petroleum ether, chloroform and ethanol extract at the dose level of 500mg/ kg of body weight has showed significant (p<0.01) 3.00±0.20, 7.00±0.50 and 12.33±0.97 abortifacient activity respectively in all the three doses when compared to control group. The abortifacient activity induced by petroleum ether, chloroform and ethanol extracts of *P. oleracea* L in female albino rats was observed by the presence of placental scars and placentomas in the uterus of the female albino rats.

The abortifecient activity of the various extract of *P. oleracea* L is mainly due to its estroogenic activity which imbalances the required progesterone and estrogen ratio Sindgi, (1975) has observed that the high dose of estrogen impropriionate to progesterone leads to resorption of fetuses.
The various extracts *P. oleracea* L administration at the dose levels of 250 and 500 mg/kg of b w caused significant resorption of fetus/embryo in the rats, which received the treatment from 8 to 14 day of pregnancy. The fetal loss in the present investigation is mainly due to the resorption of embryos as there is no vaginal bleeding observed. The persistence of placentomas in the uterus of rats is observed on day 20 of pregnancy also supports that the fetal loss is mainly due to resorption and not due to absorption. It is also stated by Greenwald and co-workers (1973, 1974) that prolactin with FSH or Estrone forms the luteotrophic complex during the early part of pregnancy. It is also proved that FSH and LH along with prolactin act as strong luteotropic complex from 8 to 14 of pregnancy in rats (Ajika *et al.*, 1972; Beatti *et al.*, 1973). The pregnancy has been interrupted in the present study as observed by ELISA test. The ineffectiveness of remaining extracts at both the dose level in causing abortion or fetal resorption may be due to their failure in inhibiting the pituitary gonadotropins and prolactin effectively (Sindgi and Rao, 1982). It is investigated that high doses of hydroxyprogesterone administered to rats' leads to dissolution of implants and death of newborn litters (Shivalingappa *et al.*, 2001). Therefore, another reason for the resorption of fetuses and appearance of placentomas in the rat’s that received ethanol extract/fraction of *P. oleracea* L may be due to the imbalanced estrogenic and progestogenic effect of the extract.
ANTIFERTILITY STUDIES OF ISOLATED FLAVONOID FROM *P. OLERACEA* L IN FEMALE ALBINO RATS

**Anti-ovulatory activity**

The estrous cycle of mammals reflects the changes in the ovary, uterus, adrenal and vagina. The cyclic changes that occur in these organs are under the synergistic influence of many hormones and factors. Any abnormality or dysfunction of these organs or their synchronization directly affects the reproductive phenomenon in the particular individual. In our study it is demonstrated that the flavonoid isolated from the ethanol extract of *P. oleracea* L has shown the prolongation of proestrus and estrus phases and reduction in number of ova in the ovary significantly compared with control rats. Many researchers have studied on the antiovalation effect of plant extract and investigated the antifertility property of Neem leaves, seeds, bark, and oil (Updhyay *et al.*, 1990; Shakti *et al.*, 1990; Riar *et al.*, 1991; Kasturi *et al.*, 2002).

**Ovarian changes**

The estrus cycle is under the controlled condition of the time sequence of various events which occurring during entire reproductive cycle, particularly the “critical period” for the release of pituitary luteinizing hormone (LH) on the afternoon of proestrus (Kobayashi *et al.*, 1969). This LH is responsible for the initiation and differentiation of follicular elements towards ovulation. During the process of differentiation, unfavorable conditions bring dedifferentiation of follicles, blockade of LH release and inhibition of ovulation in the ovaries. Estrogen secretion declines a few hours prior to the ovulatory surge of gonadotropins (Hori *et al.*, 1970). In the present investigation the administration
of flavonoid of ethanol extract has shown high estrogenic effects in the rats this might be cause for the blockage of ovulation due to alteration in the results of FSH and LH from pituitary. In our study change were observed in the level of LH, FSH, Progesterone, Estradiol and Prolactin level with the treatment of flavonoid extracts from Portulaca oleracea L. The FSH and LH were decreased significantly and the prolactin, progesterone and estradiol level is increased in diestrus stage significantly after the administration of flavonoid at high dose. The results of the present study is comparable with the studied made by Ganguly et al., (2007) who have reported that there is decrease in the FSH and LH and increase in the progesterone estradiol, and prolactin content was observed with the treatment of root extract of Mimosa pudica.

**Gravimetric & Histometric changes in ovary**

The ovary is an aggregate of mainly’ three endocrine tissues: the stroma, follicle and the corpora lutea. The healthy functioning of these tissues constitutes the net weight of the ovary, weight of the ovarian tissue increases under the influence of gonadotrophic and steroidal hormones (Peters and McNatty, 1980). The decrease in the weight of ovaries in flavonoid compound treated rats indicates the decrease in the activity of stroma, follicles formation and corpora lutea, which indicates the non-availability of gonadotrophins. In the present study, the decreased levels of FSH and LH observed by ELISA in the animals treated with flavonoid of ethanol extract supports the above view.

Follicle development is a complex and dynamic process requiring the coordinate interactions of multiple intragonadal and extragonadal factors (Richards, 2001; Richards et
al., 2002). Follicles, which contain ova, are the functional units of ovaries. The decrease in
the number of Graafian follicles in the groups, which received flavonoids of ethanol
extract, indicates that there is a disruption in their growth and differentiation (Peters and
McNatty, 1980; Knobil and Neil, 1994; Guyton and Hall, 1996; Chabbart-Buffet and
Burchard, 2002).

Pituitary hormones as well as growth factors and steroids derived from the gonads
play key role in regulating specific aspects of folliculogenesis (Wassarman&Albertini,
1994). The basic functional unit of reproduction within the ovary is the follicle (Hsueh et
al., 1984). Follicles start to grow at all times and as they develop, they produce large
number of granulosa and thecal cells. The conversion of follicles to atretic state is
functional rather than a degenerative process and is considered to be integral part of
ovarian function (Hirshfield et al., 1991). Most of the follicles undergo atresia and very
few mature to ovulate among the new crop of recruited follicles during every cycle. After
the early stage of gonadotropin independence, the entire process of follicle growth
becomes dependent on the continuous presence of gonadotropins (Hodgen, 1989; Scott
&Hodgen, 1990). Evans et al. (1997) have shown that the ovarian androgen and inhibn
secretion by follicles may play an important part in the regulation of FSH secretion and
follicular dynamics. The integral role in the control of ovarian function is played by the
hypothalamic-pituitary unit. Functioning in a coordinated manner with appropriate signals
provided by ovary via pituitary gland is responsible for the synthesis and storage of
gonadotropins-LH and FSH. These glycoprotein hormones in turn play a key role as
regulators of follicular-genesis. The decrease in the number of Graafian follicles, increase
in the number of atretic follicles and decrease/absence in the number of corpora of lutea in the ovaries of flavonoid treated animals compared to control group clearly indicates that the growth, differentiation and ovulation are inhibited due to the effect of flavonoid treatment which might have resulted in loss of hormonal receptors (Guyton, 1996).

**Biochemical changes in ovary**

Protein is considered to be the building material and is responsible for growth of organ. In the present study the low protein content of the ovary indicates the retarded ovarian growth. It is well understood that FSH is essential for protein synthesis in gonads (Means, 1975). The inhibited pituitary FSH release in flavonoid extract treated rats might have resulted in low protein content. Glycogen is involved in providing energy to various processes like ovulation, transportation and survival of eggs and implantation (Walaas, 1952). For the decreased ovulatory index and inhibiting of implantation lowered availability of glycogen may also be one of the factors. Cholesterol derived from the different sources is the precursor for the steroidogenesis of ovarian endocrine tissues (Rajendran et al., 1983). The increased ovarian cholesterol in the present study could be attributable to a probable alteration in its synthesis of steroids or transport to gonads. It is well established that biosynthetic capacity of the ovary is influenced by FSH, LH and prolactin (Hardy et al., 1974; Purandare et al., 1974; McNatty et al., 1975b). As FSH and LH levels are reduced in the rats those received the flavonoid of *Portulaca oleracea* L, the cholesterol, a precursor level is increased. Similar results were obtained by administration of extracts of *Crotalaria juncea* to rats (Vijaykumar et al., 2004).
Reactive oxygen species (ROS) play multiple roles during ovulation and reproductive process. Those such as super oxide appear to be essential in the chemical process of ovulation. ROS are thought to be released in connection with follicle rupture. Inhibition of ROS actually hinders ovulation (Miyazaki et al., 1991). The concentration of glutathione reducates may be increased in the ovaries of with flavonoid of P. oleracea L treated rats might also be one of the reasons for decreased number of ovulation (Kaneko et al. 2001).

**Uterine changes**

Estrogen influences the proliferation of stromal and epithelial cells during follicular phase. The progesterone influences secretary development, increased vascularity and deposition of lipid and glycogen especially in the endometrium. The scarcity of ovarian hormones causes underdevelopment/degneration of endometrium and myometrium (Csapo et al.. 1965; Nalbandov, 1973; Guyton and Hall, 1996).

**Gravimetric and Histometric changes of uterus**

The results of gravimetric and histological changes of flavonoid of ethanol extract of *P. oleracea* L uteri of treated rats are compared to that of control group are not in accordance with the ovarian hormonal changes. Instead of decreasing in the weight of uteri and the thickness of its sub-organs, increase in uterine weight, thickness of sub-organs in the compound treated rats is observed. This increase indicates the estrogeneric effect of the isolated flavonoid of ethanol extract. The possibility of these changes may be due to the progesteronic and estrogenic effect of the flavonoid of ethanol extract as uterine
growth and secretion depends on the availability of ovarian steroid hormones (Sindgi, 1975; Jalikhani, 1980; Findley, 1994). The estrogen primarily acts upon the surface epithelium and the glands within endometrium. Progesterone acts on estrogen-primed uterus and prepares the uterine epithelium from proliferative to secretary state (Jalikhani et al., 1980; Patil, 1988). Therefore, the ethanol extract fraction of Portulaca oleracea L is having estrogenic effects.

### Biochemical changes in uterus

Administration of flavonoid of Portulaca oleracea L to adult female rats has provoked a significant increase in the protein concentration, glycogen content and cholesterol level of the uterus. In the present study suggest that the isolated compound possess estrogenic activity, which substantiates previous findings of other investigators in immature and mature ovariecetemised rats (Mathur et al., 1987). Several studies indicates that principle effect of estrogen on carbohydrate metabolism of the rat uterus is due to increased activity of the hexokinase reaction (Roskoshki and Steiner, 1967). Therefore, the increase in the uterine glycogen contents may be due to increase in the input of estrogenic substance, which consequently increases in the glycogenic process of carbohydrate metabolism. The glycogen is mainly involved in rhythmic uterine contractions (Bo and Atkinson, 1952). As a result there is failure of implantation and interruption of pregnancy.

α-Tocopherol was discovered and recognized as factor essential for reproduction (Evans and Bishop, 1922). It is recognized as a major lipid-soluble chain breaking antioxidant found in cellular membranes and is known as an important factor in the
protection of polyunsaturated fatty acids against peroxidative damage (Poranen, 1996). This may be the reason for increased cholesterol and steroidogenesis.

Changes in the duration of estrous cycle

On the basis of cytological observations of the vaginal smears the different phases of the estrous cycle can be decided unambiguously in lower mammals like rodents, which in turn helpful to predict the effect of other factors that influence the structure and function of the ovaries. Cyclic changes in the vaginal smear observed give a reasonable index of the ovarian activity and its steroidal hormone synthesis. The levels of these hormones (Estrogen and progesterone) are controlled by pituitary gonadotropins and in turn by hypothalamic releasing hormone (Lerner, 1969). The cornification in the vaginal epithelial cells is mainly due to high levels of estrogens secreted by the matured follicles. It is also known that the exogenous administration of estrogen consistently stimulates the proliferation of the vaginal epithelium in adult spayed animals (Mandle, 1951; Boettiger, 1946).

Normally in the rats, estrogen level increases during estrous phase and decreases gradually during diestrous phase (Michel et al., 1969; Smith et al., 1975). The progesterone hormone is low during estrous phase and high during diestrous phase and highest during the proestrous phase (Smith et al., 1975). In the present investigation the increase in the duration of estrous and proestrous phases in the treated rats indicates the induced estrogenicity upon administration of the flavonoid of ethanol extract. The significant proliferation of vaginal epithelial cells during proestrous phase in treated rats
might be due to surplus availability of estrogen and progesterone in required concentration to pass to the next phase of the cycle. Moreover the ethanol extract of flavonoid of *P. oleracea* L possesses estrogenic effect as already evidenced by its ELISA method. The disruption of continued estrous phase due to estrogenicity of the extract during 15 days of experimental period may be due to the progestrigenic effect.

5.5.2. Estrogenic/anti-estrogenic activity

Many steroidal and non-steroidal plant constituents show estrogenic activity. The most potent naturally occurring estrogen is 17 β-estradiol followed by estrogen. One of the first non-steroidal estrogens is diethylstilbestrol, which is structurally similar to estradiol.

The search of potent and orally active natural products with estrogenic/anti-estrogenic activity is a milestone in the development of effective endocrine therapy. Exploration of natural products reveals that there are number of plants which possess the potentiality of preventing implantation or may cause fetal resorption. Although their exact mechanism for antifertility action is not fully understood, these agents elicit effect through variety of actions. Extracts of number of plants are known to possess estrogenicity, which increase in contractility of the smooth muscles of uterus along with increase in vascular permeability. These changes may force to expel the fertilized eggs without making any contract with the uterus ((Pathak and Prakash, 1989; Bennet *et al*, 1966).

In the present study the flavonoid of ethanol extract of *P. oleracea* L is exhibited estrogenic activity as shown by significant increase in uterine weight, diameter of the uterus, thickness of the endometrium and height of endometrial epithelium and vaginal
cornification in immature female rats when compared to control group. The treatment also caused significant increase in uterine content of glycogen, cholesterol and protein, the estrogen known to stimulate the above contents in uterus, thereby changing the uterine milieu and creating non receptive condition in the uterus (Psychoyos, 1966; Praksh 1980).

With this result the fertilized eggs do not get stable equilibrium of estrogen and fail to invade luminal epithelium. The non-steroidal compounds with estrogenic activity such as flavones, isoflavones, alkaloids, phenolics and fatty acids occur naturally in a variety of plants. There are few plants whose extracts elicit their action through the anti-progestational activity but they also possess other additional activity like estrogenic and hence their antifertility mode of action remains uncertain (Shulka et al., 1989; Prakash et al., 1985).

The results of present study also correlates to the study of Hiremath et al., (2000) who have reported that oral administration of Apigenin and Luteoline in immatured ovaractomised rats caused significant increase in weight and diameter of uterus, thickness of endometrium compared to control rats.

Phytoestrogen stimulate the growth and development of the uterus and other sexual organs. The observed changes like increase in the weight of the uteri, histological changes of flavonoid of ethanol extract treated rats clearly indicates estrogenic nature of the flavonoid (Hardman et al., 2002). As already discussed the flavonoid of ethanol extracts of P. oleracea L has high estrogenic activity it has imbalanced the hormonal
profile due to flavonoid extract treatment. Hence, anti-implantation/Abortifecient activity of plant extract may be due to imbalanced hormonal profile.

TOXICITY STUDIES OF FLAVONOID ISOLATED FROM P. oleracea L

Phytotoxicity

In the present study the isolated flavonoid from P. oleracea L at different dose level tested against germination, radical and shoot elongation of green grams used. Treatment of flavonoid of P. oleracea L at various concentrations such as 0.25, 0.5 and 1.0 mg/mL were caused significant (p<0.01) decreased in the growth between the shoot and root lengths of the 1.0 mg/mL flavonoid treated green gram seeds compared to control seedlings. The germination of seeds has occurred faster in the control relatively to those treated with flavonoid, indicates that the flavonoid of P. oleracea L is inhibited the seed germination. Reduction in the growth of root and shoot length due to flavonoid treatment proves that the green gram seeds were more susceptible to the phytotoxic effect of the compound. The available literature reports known that flavonoids as allelochemicals. Moroz and Komissarenko (1983) studied about 44 phenolic compounds these flavonoids inhibited radish germination and radical growth increase and wheat. Kaempferol, diosmethyl, phloridzin, rutin, morin, and quercetinpentaacetate stimulated radish germination and inhibited the radical growth of cress. Shalaby, (2001) reported that the flavone, chrysin, and the flavanone, hesperetin, significantly inhibited germination and hyphal growth at all applied concentrations on vesicular arbuscularmycorrhiza glomusmosseae and alfalfa plants. Flavonols isolated from leaves of Pluchealan ceolata were tested at a concentration of 10-4M and 10-3M against asparagus bean seedlings,
Phytochemical and Pharmacological activities of Portulaca oleracea L in Albino rats

resulting in an inhibitory activity (Dakshini et al., 1994).

Basile et al., (2000) have reported that the Castanea sativa Mill. Leaves contain the flavonoids quercetin, rutin and apigenin that inhibited seed germination and epicotyl and root growth in R. sativus. Some flavonoids are potent inhibitors of energy metabolism, blocking mitochondrial and chloroplast functions. Moreover, these compounds are considered as potent allelochemicals inhibiting the mitochondrial oxygen uptake. Flavonoids appear to act primarily as germination and cell growth inhibitors, possibly through interference with the energy transfer system within the cell. Flavones have been shown to interfere with ATP formation in plant mitochondria. Specific structural requirements for particular flavonoids to act as stimulators of destruction of indoleacetic acid via IAA oxidase, which results in the inhibition of ATP formation, were reported. The arrangement of the B-ring in the flavonoid structure has been proposed as responsible for the biological activity. Furthermore, in the intracellular medium, flavonoids assume a negative charge at neutral pH (Macias, et al., 1997; Martinez-Flórez et al., 2002; Tsanuo, et al., 2003; Parvez, et al., 2004; Beninger et al., 2005; Bais et al., 2006; Rolim del Almeida, et al., 2007 and 2008).

In low concentrations, these compounds can promote cellular growth, perhaps due to more effective utilization of cellular enzymes, proteins and electron carriers. high concentrations of flavonoids, on the other hand, could act as membrane hyperpolarizers, altering the ATP pump, making the flavonoids toxic for the cells, and thereby reducing their growth (Parvez, et al., 2004; Rolim Almeida, et al., 2008). Therefore, the growth
inhibition after treatment of flavonoid in our study may be due to their ability to interfere with enzyme activity.

**Zoo toxicity**

The determination of the safety of drug and plant products for human use, toxicological evaluation is carried out in various experimental animals to predict toxicity and to provide guidelines for selecting a “safe” dose in humans. The highest overall concordances of toxicity in animals with humans are with hematological, gastrointestinal and cardiovascular adverse effects (Ogbonnia et al., 2010), with certain adverse effects in humans, especially hypersentivity and idiosyncratic reactions, and are poorly correlated with toxicity observed in animals. Furthermore, it is quite difficult to ascertain adverse effects in animals such as headache, abdominal pain, dizziness, and visual disturbances. In addition, interspecies differences in the pharmacokinetic parameters make it difficult to translate some adverse effects from animals to humans.

Administration of flavonoid extract of *P. oleracea* L at the dose level ranging from 100-1000 mg/kg of b w for 2-3 days in the present study has shown no death of animal recorded, either in the experimental group or in the control group it indicates no lethality is caused by drug administration. Further, the animals did not show any change in general behavior, skin effects, defecation, loss of hairs or other physiological activities. The results of present study can be correlated to the results of Raphael et al., (2014) in their study the toxicity effect of flavonoid rich fraction of *Monodoratenuifolia* seed extract did not show any mortality during the observation period of 48 h.
The experimental rats have shown normal range of hematological parameters such as WBCs, RBCs, hemoglobin content and hematocrit values etc. This indicates that the isolated flavonoid of *P. oleracea* L has no toxic effect on physiology of experimental rats which are used in the present study. Hence, the compound is non toxic and has no toxic effect on life at the lower dose and it is safe.

**Microbial toxicity**

The isolated flavonoid of ethanol extract of *P. oleracea* L has subjected to evaluate microbial toxicity on both gram positive and negative bacterial strains by broth dilution assay method. Based on the results, obtained, the flavonoid of *P. oleracea* L it is observed that the plant extract has antibacterial activity at 40 mg/mL of concentration when compared to control group. This may be due to the chemical nature, cell membrane permeability and other factors of the isolated flavonoid. Similar results are obtained in the study conducted by Pongsak and Parichat (2010) who have reported on antibacterial activity of flavonoids extracted from leaves of *Psidium guajava* on both gram positive and negative bacterial strains. They confirmed that the antibacterial property exhibited by this plant is due the presence of flavonoid quarcitine, morin-3-O-arbinoids.

Flavonoids are most effective agents on antimicrobial activity due to their ability to complex with extracellular and soluble protein and to complex with bacterial cell wall, more lipophilic, they also disrupt microbial membrane (Tsuchiya *et al.*, 1996).
Cytotoxicity

The present study was undertaken to evaluate the cytotoxic activity of flavonoid isolated from *P. oleracea* L. and the results obtained in the present study reveals that flavonoid has not shown any significant cytotoxicity effect at the tested dose levels, indicating the nontoxic effect and safe.

Cell type cytotoxic specificity of plant extracts is likely to be due to the presence of different classes of compounds in the extract, as it has been documented in the case of known classes of compounds (Cragg *et al.*, 1994). The results of the present study indicate the presence of cytotoxic activity in some of the plant extracts from both groups (plants used to treat cancer and cancer related illnesses and those used to treat other diseases). While 37% of the plants for non-cancer disease purposes showed pronounced activity (B25% cell proliferation), only 22% of the plants for cancer diseases exhibited the same activity at the 100mg/mL concentration. Due to the fact that there were only few active plants extracts in the group of plants that are used to treat cancer diseases and that these plant extracts were only active at the highest concentration tested (100 mg/mL). Misdiagnosis of cancer by traditional healers might explain the observed lack of correlation between the reported anticancer activities of plant extracts and their cytotoxic activity on the tested cell lines. In addition, it is known that some anticancer agents might exhibit their antitumor activity *in vivo* but with no in vitro cytotoxic activity, a phenomenon that has been reported to be due to immune modulation by the compound which could lead to antitumor activity *in vivo* studies (Rosskopf *et al.*, 1992).
Genotoxicity

Evaluation of DNA damage by flavonoids of *P. oleracea* L was made by comet assay. The animals which are treated with flavonoids of *P. oleracea* L at the dose of 100 mg/mL/ kg of b w has shown significant (*p*<0.01) increase in comet tail length formation compared with controlled group. However the treatment of flavonoids of *P. oleracea* Lat 50 mg/mL/kg of b w has shown moderate and non-significant increase in comet tail length formation, when compared with control group.

The positive result obtained in gene toxicity assay of the present investigation can be correlated to the presence of flavones with certain hydroxylation patterns (5, 7 hydroxyle substitution) such as quericetin and kaempferol (Macgregor and Wilson, 1988; Vargas et al., 1989, 1990).

CHARACTERIZATION OF THE ISOLATED COMPOUND

The isolated compound of ethanol extract of *P. oleracea* L has showed maximum antifertility activity this compound is further spectral studies. The studyof LC-MS, FT-IR, and 'H NMR data of the compound suggest the possible presence of glycosidic flavonoidpresent in the sample. From the above spectral data it can be confirmed the presence of Apigenin. The structure of this molecule also supports further possible of presence of Apigenin in the isolated sample.