1. INTRODUCTION

1.1 General
Nature has innumerable and fascinating manifestation, including natural products. All the cultures, from the prehistoric times to the present day have used plant, animals and minerals as the sources of medicines. The historical use of plant for the treatment of various ailments in different civilization has resulted in development of several well organised systems of medicines. In India “Ayurveda” and in China the “Bantu” systems have been practiced since centuries. These systems, though developed almost independently, are very close to each other in concept and practice. The therapeutic properties of large numbers of medicinal plants were recognized and documented. With improved isolation and spectroscopic techniques a growing number of active constituents are being isolated and characterized from plants. Some more active chemical constituents were obtained either by chemical synthesis or analogue synthesis of the major constituents.

1.1.1. Family Iridaceae: Iris is a perennial plant growing from creeping rhizomes (rhizomatous Irises) in temperate region (1000-3000 m altitude) or, they can also grow in drier climates, from bulbs (bulbous Irises).

1.1.2. Traditional and Economical Importance of Genus Iridaceae
Several cultures have used species of Iridaceae as food, ornamental, condimental, or medicinal plants. The Navajo, the largest Native American tribe of North America, used decoctions of Iris missouriensis as an emetic (Vestal and Paul, 1952). Pieces of the rhizome of the same species were used to relieve toothaches (Nickerson and Gifford, 1966) or earaches. (Train et al., 1941). The mashed roots of Iris versicolor were applied to wounds, presumably as an antiseptic (Raymond and Marcel, 1945) and the infusions of dry roots of the same species were used to calm pain. Sisyrinchium acre was used in Hawaii in different ways. Leaves or leaf-sap were used as a dye, to give the blue color to tattoos. The use of the leaves, macerated with salt, sugar, and other spices was recommended to clean the skin and cure skin diseases.
(Speck et al., 1942). *Iris ensata* was used in India as anthelmintic and diuretic, and, mixed with other species, to treat venereal diseases (Chopra and Nayar, 1986)

**1.1.3. Species of genus Iris:**

Plants of genus *Iris* comprise over 3000 species in the world of which twelve species are reported in India (Bhattacharjee, 1998) but most commonly *Iris* species found in India are *Iris croceae, Iris ensata, Iris germanica, Iris hookerian, Iris kumaonensis* and *Iris kashmiriana* specially found in Himalayan regions. Medicinal plants belongs to this genus has been named Irsa in Iranian traditional medicine. *Iris* is a genus of variety of species of flowering plants with showy flowers.

**1.1.4. Biological activities of Iris plants:** The phytochemical screening and chemical investigations of various species of *Iris* have resulted in the isolation of variety of secondary metabolites. Approximately more than two hundred compounds have been reported from the genus *Iris* which includes flavonoids, isoflavonoids and their glycosides, benzoquinones tri terpenoids and stilbenes glycosides (Dhar et al., 1978 and 1990; Atta-ur-Rahman et al., 2000 and 2003; Choudhary et al., 2001). Many species of this genus have been used for long in medicine for their interesting biological activities. The peeled and dried rhizomes of Iris species, collectively known as rhizome iridis enjoyed popularity due to their emetic, cathartic, diuretic stimulant and expectorant properties (Steinberger and Hansel, 1988). The rhizomes of this plant have been extensively used against fever, kidney infections and as ingredients of tooth powders etc. The rhizomes of *Iris hookeriana* exhibit significant anthelminthic activity against gastrointestinal nematodes of sheep. Extracts of *Iris* species in general possess strong total antioxidant, effective reducing power and exhibit free radical scavenging, metal chelating activities and inhibition of lipid peroxidation (Nadaroglu and Demir, 1990). Powder of roots of *Iris songarica*, mixed with curd is used to cure diarrhea, (Ali, 2000). These species have been introduced as diuretic and expectorant at low doses and as a strong purgative and emetic in high doses. It has been reported that Irsa is useful for pulmonary, asthma, liver and uterus diseases as well as hemorrhoid and gripe (Mirheidar and Moaref, 1996)
1.1.5. Biological Activities of Isoflavonoids:
Isoflavonoids flavonoids and their glycoside are prominent plant secondary metabolites with variable phenolic structures and are consumed by humans as dietary constituents. Although not considered nutrients and thus essential for life, flavonoid ingestion may play a significant role in health and disease (Bhattacharjee, 1984; Steinegger et al., 1988). Numerous studies have shown an association between isoflavone-rich dietary consumption and reduced cancer risk, particularly breast and prostate cancers (Nadaroglu et al., 1985; Rastogi and Mehrotra, 1991; Williams et al., 1997). The preventive role of isoflavones in cancer (Nadaroglu et al., 1985; Hadfield et al., 2000; Marner and Hanisch, 2001), cardiovascular diseases, osteoporosis, and menopausal symptoms in addition to their antioxidant (Ali et al., 1983; Damme et al., 1997), antimicrobial (Bonfills et al., 2004), anti-inflammatory, and estrogenic activities (Rastogi et al., 1977) have largely been documented.

1.1.6. Chemotaxonomy of Iridaceae:
Classes of phenolic present include anthocyanin, flavonols, flavones, isoflavones, xanthones, biflavones, naphthoquinones and proanthocyanidins. Other groups of secondary metabolite recorded in the family are saponins, fructans, non-protein amino acids, sterols, and bufadienolides. Some individual metabolites are of economic importance. The sesquiterpene α -irone from Iris florentina L. is valued for its characteristic scent of violets and is used in perfumery. The yellow carotenoid-like pigment crocein from styles of Crocus sativus L. is employed as a food colorant. Again, the isoflavones iridin is a major component of orris root, obtained from the rhizome of Iris florentina, and still used in perfumery. Here, attention will be mainly focused on the more recent survey of phenolic patterns in the family, which covered 255 species from 57 genera (Williams et al., 1986). Some general conclusions relating these phenolic profiles with tribal classification will be mentioned later. In the meantime, the systematic implications of the distribution of individual subclasses of phenolic will be considered. The subclasses considered here are the, isoflavones, xanthones, biflavonoids, anthocyanin and quinones. In present work, the emphasis would be given to isoflavones.
1.1.7. Isoflavones:

The presence of isoflavones in rhizomes or aerial parts is one of the most distinctive chemical features of the genus Iris. One of the earliest reports on this genus was of the isoflavones irigenin and its 7-glucoside iridin in orris root, Iris florentina. Their chemical structures were determined by (Baker, 1928). Orris root is a preparation of the powdered rhizome. Iridin is a major component and must contribute to the use that is made of orris root in perfumery. Since then, irigenin and/or iridin have been isolated from at least nine other species. These are I. germanica L., I. kamaonensis Wall. Ex G. Don, I. marsica, I. lutescens Lam., I. nepalensis Wall., I. pallida Lam., I. setina Colas., I. tingitana Boiss. Reut. and I. unguicularis Poir.

The isoflavone irigenin has a unique substitution pattern, which is rarely observed outside Iris. Within Iris, irigenin is frequently accompanied by one or more related structures but particularly by tectorigenin, first obtained from I. tectorum Maxim. Some thirty other isoflavones have been described variously from Iris species. A recent review described several new structures from Iris japonica Thunb (Aerial parts), I. nigricans Dinsm (rhizomes) and I. kashmiriana Bak (rhizomes) (Boland and Donnelly, 1998). Isoflavones have been reported from two other genera in Iridaceae, Belamcanda chinensis and Patersonia, but otherwise appear to be absent from most species in the family (Williams et al., 1986).

According to Ingham’s review (1983), the rhizome of Belamcanda Adans contains tectorigenin 7-glucoside and iridin. This occurrence is very expectable, since this plant has always been regarded as being closely related to Iris; the report of tectorigenin and iristectorigenin A in Patersonia occidentalis R. Br.var. angustifolia is more surprising. Isoflavones appear to be rare in Patersonia, however, and they could not be detected in the closely related subspecies with broad leaves, P. occidentalis, var. occidentalis (Williams et al., 1989). Finally, it should be emphasized that isoflavones, while occurring in the Leguminosae and at least a dozen other dicotyledonous families, are relatively rare in the monocotyledons. There are only two reports outside the Iridaceae, in Festuca L. (Gramineae) and Hemerocallis L (now Hemerocallidaceae) (Dewick, 1994).
The wide spectrum of pharmacological activities associated with the isoflavones prompted us to undertake the phytochemical investigation of *Iris kashmiriana*, which is reported to be a rich source of isoflavones. (Dhar *et al.*, 1978, 1990, 1996; Shibli and Ajlouni, 2000).
The present work deals with the isolation and structure elucidation of two glycoside isoflavones from the rhizomes of *I. kashmiriana*. Out of two one isoflavonoids is completely new. The new isoflavones have been subjected for different analogue synthesis with marked cytotoxic activity on different cell lines. In summary, there is no doubt that the *Iridaceae* contain a heterogeneous collection of flavonoid and related phenolic constituents. Many of these are potentially useful as taxonomic markers, but further detailed surveys are still needed to establish whether or not they are important for the classification of this diverse family.

**Biological Source:** *Rhizome of Iris kashmiriana.*

**Common Name:** Mazarmund.

**Synonyms:** Juno, Junopsis, Pardanthopsis

**Isoflavones as anticancer agents:** The structure of isoflavones closely resemble with potent natural anticancer agents like colchicine, combretastatin, phenstatin, in these molecules the two phenyl rings are separated by three, two and one carbon atoms respectively. They have shown anticancer activity by means of inhibiting microtubules at G2/M phase (Downing *et al.*, 1999). The isoflavones would probably follow the same mechanism of action against different cancer cell lines.

![Fig No.1.1.7.6 Isoflavone](image1)

![Fig No.1.1.7.7 Phenstatin](image2)

![Fig No.1.1.7.8 Combretastatin](image3)
1.2. Aim and Objectives:

1.2.1. Aim: Isolation, Characterization and Biological Evaluation of Bioactive Isoflavone from Iris kashmiriana.

1.2.2. Objectives

1) Extraction and isolation the bioactive molecules from Iris kashmiriana.
2) Phytochemical screening were performed to identify the presence of secondary metabolites
3) Purification and Characterization of the isolated compounds and their analogues were done by melting point determination and TLC. Analysis (Rf value data.)
4) Qualitative estimation of Isoflavone and its analogue were performed by RP-HPLC.
5) Structures of the isolated molecules and their analogues were established by U.V. IR, H\(^1\)NMR, C\(^{13}\)NMR, and 2-D NMR spectroscopy and mass spectrometry.
6) Structures were also established by U.V. shift to locate the position of hydroxyl groups in isolated molecules.
7) All the isolated compounds and their analogues were subjected to following biological activities.
   i) Antioxidants
   ii) Antimicrobials
      a) Antibacterial    b) Antifungal.
   iii) Cytotoxic activity on different cell lines.
      iv) Cell Cycle Analysis of selected compounds using Flow Cytometer.

1.2.3. Rationale of the study :

- Iridaceae is the only family of the monocot having highest numbers of isoflavones and their glycosides.
- This family is having a wealth of chemical structures, specifically of the phenolic type and thus, probability of finding new molecules was significant.
- The anticancer study along with the cell cycle analysis was not explored yet.
• The traditional and folk use of the plant of this family gives the idea of diverse and prominent nature of chemical structure as well as their pharmacological activities. Like anticancer, antimicrobial and antioxidant, anthelminthic etc.

• The wide spectrum of pharmacological activities associated with the isoflavones prompted us to undertake the phytochemical investigation of *Iris kashmiriana*, which is reported to be a rich source of isoflavones.

• The structure of the isoflavones closely resembles with natural products which are very potent anticancer agents. Isoflavones also fit into the “Butter fly” model for anti-cancer agents. eg. stilbenes, diazdine, genacitin, phenastatin etc.