'Equipped with his five senses, man explores the universe around him and calls the adventure Science.'

~Edwin Powell Hubble
2.1 Rational

Plant derived substances (secondary metabolites) have recently become of great interest owing to their versatile applications in curing many diseases. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube et al., 2008).

Extraction is the separation of medicinally active portions of plant using selective solvents through standard procedures. The products obtained from plants are relatively complex mixtures of secondary metabolites, either in liquid or semisolid state or in dry powder or crystalline form and can be used for oral or external use for therapeutic usage. These include classes of preparations known as decoctions, infusions, fluid extracts, tinctures, pilular (semisolid) extracts or powdered extracts. Such preparations have been popularly called galenicals, named after Galen, the second century Greek physician (Remington et al., 2008).

The pharmaceutical industries, involves the extraction procedure for the separation of medicinally active portions of plant tissues from the inactive/inert components by using selective solvents. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity (Ncube et al., 2008).

The purpose of standardized extraction procedures for crude drugs (medicinal plant parts) is to attain the therapeutically desired portions in large amount and to eliminate unwanted material by treatment with a selective solvent known as menstrum. The extract thus obtained, after standardization, may be used as medicinal agent as such in the form of tinctures or fluid extracts or further processed to be incorporated in any dosage form such as tablets and capsules. These products contain complex mixture of many
medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, flavonoids and lignans (Tiwari et al., 2011).

There are certain general techniques of medicinal plant extraction used in different areas such as pharmaceutical industries, herbal industries research laboratories include maceration, infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet), aqueous-alcoholic extraction by fermentation, counter-current extraction, microwave-assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction, and phytonic extraction (with hydrofluorocarbon solvents). Preparation of aromatic plants extract also include some extraction methods such as hydro distillation techniques (water distillation, steam distillation, water and steam distillation), hydrolytic maceration followed by distillation, expression and effleurage (cold fat extraction) may be employed. Some of the latest extraction methods for aromatic plants include headspace trapping, solid phase micro-extraction, protoplast extraction, microdistillation, thermo-microdistillation and molecular distillation (Tiwari et al., 2011).

The basic parameters influencing the quality of an extract are (Ncube et al., 2008).

1. Plant part used as starting material
2. Solvent used for extraction
3. Extraction procedure

Effect of extracted plant phytochemicals depends on (Ncube et al., 2008).

1. The nature of the plant material
2. Its origin
3. Degree of processing
4. Moisture content
5. Particle size

The variations in different extraction methods that will affect quantity and secondary metabolite composition of an extract depend upon (Ncube et al., 2008).
Successful determination and extraction of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extractions includes low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, and inability to cause the extract to complex or dissociate. The factors affecting the choice of solvent are quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process, and the potential health hazard of the extractants (Eloff et al., 1998). The choice of solvent is influenced by what is intended with the extract. Since the end product will contain traces of residual solvent, the solvent should be non-toxic and should not interfere with the bioassay. The choice will also depend on the targeted compounds to be extracted (Ncube et al., 2008; Remington et al., 2008; Handa et al., 2008; Das et al., 2010; Tiwari et al., 2011).

Secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of plants (Fraenkel, 1959). The absence of secondary metabolites does not result in immediate death like primary metabolites; secondary metabolites often play an important role against plant defense herbivory and other interspecies defenses (Stamp, 2003).

### 2.2 Review of literature

The survey of literature revealed that the isolation of a large number of bioactive compounds from different medicinal plants is useful in the treatment
of various diseases. The pharmacological studies have provided several evidences that fractions of medicinal plant are found to be very effective in many important ailments. The studies also provided the evidence that the isolated compounds and their extracts were found to be active for curing many diseases.

It was well known fact that spices not only add flavor and color, but they also impart medicinal value to the food. The researches carried out by different scientists all over the world have very well documented the pharmacological effects of the spices. They have an array of bioactive constituents which are responsible for their medicinal and therapeutic value. Due to their high medicinal importance, these spices still need further investigations to isolate more new active compounds. The survey of literature of the spices selected for the present study i.e., *Nigella sativa* L. (Kalonji), *Piper nigrum* L. (Black pepper), *Pimpinella anisum* L. (Aniseed) and *Trachyspermum ammi* L. (Ajwain) showed the extraction of a large number of important compound used as therapeutic agents.

### 2.2.1 *Nigella sativa* L. (Kalonji)

Al-Sa'aidi (2009) studied the effect of alcoholic extract of *Nigella sativa* L. on fertility parameters in white male rats (*Rattus norvegicus*). The orally administered *Nigella sativa* L. alcoholic extract showed the chemo preventive effects, by the induction and development of 1, 2-dimethylhydrazine-induced aberrant crypt foci (ACF) and putative preneoplastic lesions for colon cancer in rats. Immuno histochemical analysis of 5-bromo-2'-deoxyuridine labeling in colonic crypts revealed that *N. sativa* L. have significant anti-proliferative activity in both initiation and post-initiation stages. These findings demonstrated that the *N. sativa* L. alcoholic extract has the ability to inhibit colon carcinogenesis of rats in the postinitiation stage with adverse side effects and that the inhibition may be associated with suppression of cell proliferation in the colonic mucosa. It was also reported by Farah and Begum (2003) that *N. sativa* L. alone or in combination with oxidative stress was found to be effective in vitro in
inactivating MCF-7 breast cancer cells, unveiling opportunities for promising results in the field of prevention and treatment of cancer.

In many countries, black seed oil is used as a natural remedy for a wide range of diseases including various allergies. The plant's mechanism of action is still largely unknown (Kalus et al., 2003). Le et al., (2005) studied the in vivo treatment of petroleum ether extract of Nigella sativa L. it was concluded that it exerts an insulin-sensitizing action by enhancing the activity of the two major intracellular signal transduction pathways of the hormones receptor.

The crude extracts and essential oil of N. sativa L. were proved to have many therapeutic effects. The N. sativa L. alcoholic extract was found to be as effective as metronidazole in the cure of giardiasis (Bishara et al., 1992). Moreover, aqueous extract has demonstrated inhibitory effect against candidiasis (Khan et al., 2003) and a potential therapeutic effect against Blastocystishominis (El Wakil, 2007).

The alcoholic extract of N. sativa L. showed a lowering of blood pressure (Zawahry 1963). Hypotensive and antispasmodic glycosides and hypertensive alkaloid were isolated from N. sativa L. (Zawahry 1963, Zawahry and Kararra 1964).

2.2.2 Piper nigrum Linn. (Black pepper)

The black pepper extract showed the effect on corrosion inhibition on mild steel (MS) in 1 M H₂SO₄ media was evaluated by conventional weight loss studies. Results of weight loss study reveal that black pepper extract acts as a good inhibitor even at high temperatures also (Raja et al., 2008).

The respiratory tract sensations are important in alleviating smoking withdrawal symptoms. Cigarette substitutes delivering pepper constituents may prove useful in smoking cessation treatment (Rose et al., 1994). Western blotting revealed the down-regulation of SIRT1 protein expression in Daudi cells treated with extracts of black pepper. The effect on the SIRT1 gene expression examined by reverse transcription polymerase chain reaction was
unaltered. It was concluded that component of black pepper may induce the down-regulation of SIRT1 protein (Nishimura et al., 2011).

The methanolic extract of *P. nigrum* L. fruits has hepatoprotective and antioxidant effects in rats (Singh et al., 2007). Singh and Rao (1993) reported the potential of *P. nigrum* L. on the hepatic biotransformation system in mice. The level of MDA was lowered in the group fed on 2% black pepper diet for 20 days. Being a potential inducer of detoxication system, the possible chemopreventive role of black pepper in chemical carcinogenesis is suggested.

An alkaloid piperine was extracted from dry seeds of plant *Piper nigrum* L. with ethanol by using soxhlet extraction then isolation and purification by re-crystallization, the structure of Piperine was confirmed by the IR spectroscopy by Zainab (2010).

2.2.3 *Pimpinella anisum* Linn. (Aniseed)

Cardozo et al., (2006) studied the aniseed oil treatment to decreased acetate to propionate ratio, branched-chain VFA and ammonia concentrations, and protozoal counts. It was also indicated that at the doses used a mixture of cinnamaldehyde and eugenol, aniseed oil, and capsicum oil may be useful as modifiers of rumen fermentation in beef production systems.

Kassi et al., (2004) studied an estrogen receptor-related mechanism of aqueous extracts of *Pimpinella anisum* seeds, flowers of *Sideritis euboea* and *clandestina* and *Matricaria camomilla in vitro* and were found to be active in stimulating the differentiation and mineralization of osteoblastic cell culture and inducing the anti-estrogens, the insulin growth factor binding protein 3 (IGFBP3) in MCF-7 breast cancer cells. The presence of estradiol inhibited the anti-estrogenic effect, thus suggesting an estrogen receptor-related mechanism.

Aniseed alcoholic extracts and oil exerted a relaxing effect on *in vitro* pre-contracted smooth muscles from different organs (tracheal and ileal) by antagonizing several contraction-inducing agents. Aniseed essential oil produced a complete relaxation of carbachol-induced contractions in the isolated tracheal smooth muscle from guinea pig. In contrast, the oil increased
the contraction force in electrically-stimulated guinea pig ileal smooth muscle (Reiter and Brandt, 1985).

Boskabady and Ramazani-Assari (2001) reported the bronchodilatory relaxant effect of aniseed essential oil, aqueous extract and ethanol extract on isolated tracheal chains (methacholine pre-contracted) of guinea pig.

An aqueous extract of a mixture of herbs including aniseed, were tested for their inhibitory effect on histamine released from rat peritoneal mast cells stimulated either by compound or by IgE/anti-IgE. The effect of the herbal extract was compared to that of the flavonoid quercetin. The herbal water-extract inhibited histamine released from chemically- and immunologically-induced cells by 81% and 85%, respectively; quercetin treated cells were inhibited by 95% and 97%, respectively (Haggag et al., 2003).

2.2.4 *Trachyspermum ammi* (Linn) Sprague (Ajwain)

Anilakumar et al., (2009) evaluated the potential of ajwain extract on hexachlorocyclohexane-induced oxidative stress and toxicity in rats. Results revealed that HCH administration lead to an increase in hepatic lipid peroxidation associated with reduction in levels of glutathione (GSH), activity of superoxide dismutase (SOD), and catalase and glucose-6-phosphate dehydrogenase. It was concluded that HCH administration resulted in hepatic free radical stress, causing toxicity, which could be reduced by the dietary ajwain extract. Seed extracts of *Trachyspermum coticum*, leaf extracts of *Lavandula angustifolia* and flower extracts of *Rheum ribes* effectively inhibited the radial growth and spore germination of *Fusarium oxysporum* fungus by using of filter paper and poisoned food methods (Ghorbany et al., 2010).
In vitro activity of a methanolic extract of fruits of *Trachyspermum ammi* L. (Apiaceae) against adult bovine filarial *Setaria digitata* worms has been investigated. It was screened for in vivo antifilarial activity against the human filarial worm *Brugia malayi* in *Mastomys coucha*, showing macrofilaricidal activity and female worm sterility in vivo against *B. malayi*. These findings thus provide a new lead for development of a macrofilaricidal drug from natural products (Mathew et al., 2008).

The crude extract of *Ajwain* produces a fall in BP and heart rate (HR) of anesthetized normotensive (NMT) rats. Hypotension produced is very brief and returns to normal within a minute. At the low dose the crude extract produces negligible change in the HR (Gilani et al., 2005).

A large number of plants such as *Cassia angustifolia* (Senna), *Carum copticum* (Ajwain), *Terminalia chebula* (Himej), *Embelia ribes* (Vidang) and *Glycyrrhiza glabra* (Jethimadh) have been reported to exhibit potent contraceptive activity in animal models and certain clinical studies (Srivastava et al., 2005).

Comparative antihyperlipidaemic efficacy of *Trachyspermum ammi* L. (Ajwain) extracts in chloroform, methanol, petroleum ether and water was investigated in albino rabbits. Petroleum ether extract appeared to be more potent than methanol extract on the basis of increasing the level of HDL-cholesterol and lowering the LDL-cholesterol more effectively than methanol extract. Petroleum ether extract reduced atherogenic index (total cholesterol/HDL-cholesterol) more effectively than methanol extract (Javed et al., 2006).

**2.3 Materials and Methods**

**2.3.1 Collection of Pant Material**

The seeds of all the four spices *i.e.*, *Nigella sativa* L. (Kalonji), *Piper nigrum* L. (Black pepper), *Pimpinella anisum* L. (Aniseed) and *Trachyspermum ammi* L. (Ajwain) were procured form the local market.
These seeds were identified by Head of the Department of Botany of G.F. College, Shahjahanpur and specimen were kept for the record with the following voucher no. *Nigella sativa* L. (Kalonji) Chem./ Biotech A1-01, *Piper nigrum* L. (Black pepper) Chem./ Biotech A1-02, *Pimpinella anisum* L. (Aniseed ) Chem./ Biotech A1-03 and *Trachyspermum ammi* L. (Ajwain) Chem./ Biotech A1-04.

### 2.3.2 Extraction Methodology

#### 2.3.2.1 Preparation of Seed Powder

The *Nigella sativa* L. (Kalonji), *Piper nigrum* L. (Black pepper), *Pimpinella anisum* L. (Aniseed) and *Trachyspermum ammi* L. (Ajwain) seeds were washed thoroughly with distilled water thrice and dried between the folds of filter paper and finally in air. The 250 gm dried seeds were then were coarsely powdered through pestle and mortar and then subjected to Soxhlet apparatus (Fig 2.1) for extraction.

![Fig.2.1: The Soxhlet apparatus](image-url)
2.3.2.2 Preparation of Extracts through Soxhlet

A little amount of glass wool was placed in thimble where the siphon arm is open, and then powdered seeds were filled into the thimble of the Soxhlet assembly. The glycerin was placed at the mouth of extraction chamber and at the mouth of condenser to avoid any leakage. The extraction chamber was then filled with Petroleum ether through the open end so that the seeds were properly dipped in it. After the completion of extraction with petroleum ether to remove fatty acids, the extraction were carried out successively by different solvents (500 ml each) of increasing polarity such as benzene, chloroform, ethyl acetate and methanol, with each solvent until the extracting solvent become colorless (Fig.2.2-2.5).

2.3.3 Concentration of different extracts of spices

After the completion of the extraction all the extracts of different solvents of *Nigella sativa* L. (Kalonji), *Piper nigrum* L. (Black pepper), *Pimpinella anisum* L. (Aniseed) and *Trachyspermum ammi* L. (Ajwain) were concentrated by vaccum distillation to get semi solid masses (Figs.2.6-2.9).

Out of these only few extracts of considerable interest was selected for further examination and subjected to column chromatography.

2.4 Results and Discussions

The weights of the different extracts of all the four spices obtained through soxhlet assembly were tabulated in the table (2.1).

The extracts of *Nigella sativa* L. in petroleum ether, chloroform and ethyl acetate contain a layer of oil over it, which is dark brown in colour. The weight of *N. sativa* L. crude extract in benzene was 14.2 gm in 500 ml following the petroleum ether (8.3gm), Chloroform (7.3gm), methanol (5gm) and ethyl acetate (3.4gm). The alcoholic extract of *N. sativa* L. showed the positive result to improve the male fertility in rats by Al-Saaidi (2009) and the petroleum ether extract is useful in the *in vivo* treatment of diabetes (Le *et al.*, 2005)

Table 2.1: Extracts (gm) extracted through soxhlet assembly of all the four spices in different solvents of increasing polarity.
<table>
<thead>
<tr>
<th>S.no</th>
<th>Extracting Solvent (500ml)</th>
<th><em>Nigella sativa</em> (gm)</th>
<th><em>Piper nigrum</em> (gm)</th>
<th><em>Pimpinella anisum</em> (gm)</th>
<th><em>Trachyspermum ammi</em> (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Petroleum ether</td>
<td>8.3</td>
<td>3.5</td>
<td>5.2</td>
<td>4.2</td>
</tr>
<tr>
<td>2</td>
<td>Benzene</td>
<td>14.2</td>
<td>3</td>
<td>2</td>
<td>3.4</td>
</tr>
<tr>
<td>3</td>
<td>Chloroform</td>
<td>7.3</td>
<td>4.5</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>Ethylacetate</td>
<td>3.4</td>
<td>3.5</td>
<td>3.6</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>Methanol</td>
<td>5</td>
<td>3.5</td>
<td>3</td>
<td>5.5</td>
</tr>
</tbody>
</table>

*Piper nigrum* L. (Black pepper), extract in ethyl acetate also contains oil which is brown in colour. The *Piper nigrum* L. extracts prove to be useful in the smoking cessation treatment (Rose et al., 1994). Singh and Rao (1993) also reported the potential of *Piper nigrum* L. on hepatic biotransformation in mice. The *P. nigrum* L. extracts were black in colour in all the four extracting solvents except in petroleum ether which is orange brown in colour. The amount of chloroform extract of black pepper is 4.5 gm in 500ml.

*Pimpinella anisum* L. (Aniseed) in petroleum ether and methanol also contains the oily extract of dark brown in colour. All the extracts of aniseed were orange brown in colour. The aqueous and alcoholic extracts of aniseed were found to be active in stimulating the differentiation of osteo-blastic cell and in relaxing the pro-contracted smooth muscles of different organs *in vivo* respectively. (Kassi et al., 2004; Reiter and Brandt, 1985)

Anilakumar *et al.*, (2009) reported that administration of Ajwain extract will reduced the HCH induced oxidative stress and toxicity in rats. *Trachyspermum ammi* L. (Ajwain) in petroleum ether obtain an orange greasy extract. The extracts of ajwain in all the extracting solvent were brownish in colour. The crude extract of ajwain produces fall in blood pressure and heart rate of anesthesitized normotensive rats by Gilani *et al.*, (2005). Petroleum ether extract reduced atherogenic index more effectively than methanol extract of Ajwain (Javed *et al.*, 2006).

The extracts of *Nigella sativa* L. (kalonji) in methanol, *Piper nigrum* L. (Black pepper) in ethyl acetate, *Pimpinella anisum* L. (Aniseed) in chloroform
and *Trachyspermum ammi* L. (Ajwain) in methanol were considered for further examination. These were subjected to column chromatography and thin layer chromatography, for the separation of pure compound of medicinal value.

### 2.5 Conclusion

It was concluded that the spices contain a huge amount of secondary metabolites, there is enough quantity of fatty acids and other metabolites. There is need of proper phytochemical investigation and extraction of secondary metabolites from the spices for the future use in the manufacturing of herbal drugs.
Fig. 2.2: Extraction of *Nigella sativa* L. (Kalonji) through soxhlet assembly
Fig. 2.3: Extraction of *Piper nigrum* L. (Black pepper) through soxhlet assembly
Fig. 2.4: Extraction of *Pimpenella anisum* L. (Aniseed) through soxhlet assembly
Fig. 2.5: Extraction of *Trachyspermum ammi* L. (Ajwain) through soxhlet assembly
Fig. 2.6: Concentrated extracts in organic solvent of increasing polarity such as Petroleum ether, Benzene, Chloroform, Ethyl acetate and Methanol of *Nigella sativa* L. (Kalonji).

Fig. 2.7: Concentrated extracts in organic solvent of increasing polarity such as Petroleum ether, Benzene, Chloroform, Ethyl acetate and Methanol of *Piper nigrum* L. (Black pepper).

Fig. 2.8: Concentrated extracts in organic solvent of increasing polarity such as Petroleum ether, Benzene, Chloroform, Ethyl acetate and Methanol of *Pimprenella anisum* L. (Aniseed).

Fig. 2.9: Concentrated extracts in organic solvent of increasing polarity such as Petroleum ether, Benzene, Chloroform, Ethyl acetate and Methanol of *Trachyspermum ammi* L. (Ajwain).
2.6 References


