Review of Literature & Scope
CHAPTER 2

REVIEW OF LITERATURE AND SCOPE OF THE WORK

The review of literature pertaining to the present study involving two medicinal plants, *Acalypha fruticosa* (Forsk) and *Aristolochia tagala* (Cham) is presented under the following headings.

2.1 *Acalypha fruticosa*-its details and therapeutic uses.
2.2 Activity studies on *Acalypha fruticosa*.
2.3 Activity studies and therapeutic uses of other *Acalypha* species.
2.4 Chemical constituents of other *Acalypha* species.
2.5 Phytochemical constituents of other *Euphorbiaceous* plants.
2.6 *Aristolochia tagala*- its details, activity studies and therapeutic uses.
2.7 Phytochemical constituents of *Aristolochia tagala*.
2.8 Phytochemical constituents of other *Aristolochia* species.
2.9 Essential oils from *Aristolochia* species.
2.10 Chemistry of flavonoids.
2.11 Scope of the work.

2.1 *Acalypha fruticosa* Forsk- its details and therapeutic uses

*Acalypha* is the fourth largest plant genus of the family *Euphorbiaceae* which consists of about 300 genera and 5000 species. Some of the popular Indian genera are *Acalypha, Euphorbia, Phyllanthus* (Indian Gooseberry), *Jatropha* (purging nut, castor).

*Acalypha* is the sole genus of the subtribe *Acalyphinae* of the tribe *Acalyphaeae* of the subfamily *Crotonoideae* within the *Euphorbiaceae* family. *Acalypha* sp., known as Copper leaf or Three Seeded Mercury, are hardy and colourful plants with various coloured leaves. It has about 250 species of herbs and shrubs. About 27 species are found...
in India of which *Acalypha indica*, *Acalypha fruticosa*, *Acalypha ciliate*, *Acalypha racemosa* and *Acalypha hispida* have wide therapeutic use.

An infusion of the leaves of *Acalypha fruticosa* is regarded as a stomachic and alternative. The leaves are prescribed in digestive troubles such as dyspepsia, colic, and diarrhea and even cholera. The leaf juice is used in opthalmia. An infusion of the leaves is used as a vulnerary to wash pustules. The root is used in gonorrhoea (Wilson *et al.*, 1962). It is used as an adulterant for *Acalypha indica* which is widely used in homeopathy (The Homeopathic Pharmacopoeia, 1941; Homeopathic Pharmacopoeia, 1971) and in folk medicine (Madaus, 1938).

### 2.2 Activity studies on *Acalypha fruticosa*

No reports on phytochemical isolation and characterization of any compound from *Acalypha fruticosa* are available. The only available literature regarding this plant deals with the screening of the plant for antimicrobial and antioxidant studies. Interesting reports come in the form of very good antibacterial, antifungal and anticancer activities.

Among the many Yemeni medicinal plants screened for antibacterial activity, *Acalypha fruticosa* showed the highest activity against two Gram-positive bacteria, *Staphylococcus aureus*, *Enterococcus faecalis* and two Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa* and a yeast *Candida albicans* (Alasbaki *et al.*, 1999). Further studies (Ranzi *et al.*, 2008) showed that the methanolic and aqueous extracts of *Acalypha fruticosa* possess significant antibacterial activity (Bauer *et al.*, 1996) against *Bacillus subtilis*, *Micrococcus flavus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus* and *Candida maltosa*. Both the methanolic and aqueous extracts showed very high antioxidant activity (Brand *et al.*, 1995) with IC$_{50}$ values of 70$\mu$g/ml and 1000$\mu$g/ml respectively. Phytochemical screening showed the presence of terpenoids, flavonoids and tannins in the extracts, which could be responsible for the above activity. The most important observation was that its extracts proved to have potential anticancer effect against 5 cancer cell-lines (Mothana *et al.*, 2007).
2.3 Activity studies and therapeutic uses of other *Acalypha* species

The Indian *Acalypha* called *Acalypha indica* showed significant antifungal (Bhowmick *et al*, 1982), antibacterial (Gopalakrishnan *et al*, 2000 and Hiremath *et al*, 1993) and antinfertility activities (Hiremath *et al*, 1999). Its contraceptive principle was reviewed (Chauhan *et al*, 2003). A tincture of *Acalypha indica* is effective for haemorrhage of the lungs. *Acalypha indica* finds use as carminative, diuretic, emetic expectorant, anthelmintic and laxative and in the treatment of asthma and bronchitis. It is beneficial in treatment of scabies, bed sores and maggot- injected wounds and other skin diseases and used to cure tooth and ear ache. The herb is a remedy for severe cough associated with bleeding from the lungs (Kithiakar *et al*, 1975, Guha Bakshi *et al*, 1972 and Saha *et al*, 1962).


2.4 Chemical constituents of other *Acalypha* species

To date, only a few representatives have been investigated chemically. The chemical investigation of some plant species of *Acalypha* led to the isolation of different classes of natural products including phenolic compounds such as flavonoids, tannins, sterols, diterpenes and amides as well as cyanogenic glycosides (Zhang *et al*, 1994; Siems *et al*, 1996; Hiremath *et al*, 1998; Yoshiaki *et al*, 1999).
The significant compounds present in *Acalypha indica* are acalyphin (1) (Adolf Nahrsted et al., 1982), an amide called acalyphamide (2), isolated as its acetate (3), aurantiamide (4) and its acetate (5), 2-methyl anthraquinone and tri-O-methyl ellagic acid (6) isolated as its acetate (6a) (Bani Talapatra, 1981). Besides these, stigmasterol, sitosterol, succinimide, kaempferol and an essential oil are present. Acalyphin has structure similar to ricinine, ricinidine (Ganguly, 1970), nudiflorine (Mukherjee et al., 1966) and mallorepine (Hikino et al., 1978) all of which were isolated from the tribe *Acalyphae*.

![Chemical structures](image)

(1) R=H
(2) R=COCH₃
(3) R=H
(4) R=H
(5) R=COCH₃
Anthocyanins acylated with gallic acid, (7, 8, 9) were isolated from *Acalypha hispida* for the first time from this family (Bergitte Reiersen *et al.*, 2003), though 3- rutinosides of cyanidin, delphinidin and pelargonidin have been reported (Asen, 1958; Stewart *et al.*, 1979; Del *et al.*, 1983). Capsules containing anthocyanins from *Euphorbia splendens* used for the treatment of blood circulation disorders have been patented (Fuji *et al.*, 1987).

(7) = cyanidin-3- O- β- galactopyranoside

(8) = cyanidin-3- O- (2′′- O-galloyl- β- galactopyranoside)

(9) = cyanidin-3-O- (2′′′- O-galloyl-6′′- O-α-rhamnopyranosyl-β-galactopyranoside)
Tannins and ellagitannins are reported from many plants of *Acalyphainae* subtribes like *Acalypha indica* (Asima Chatterjee *et al*, 2003), *Acalypha hispida* (Yoshiaki *et al*, 1999). Gallic acid, geranin and corilegin were isolated from *Acalypha hispida*, *Acalypha wilkesiana* and *Acalypha communis* (Adesina, S. K., *et al*, 2000; Maria Teresa *et al*, 2002).

Two new friedolabdanes (10, 10a) and a new curcumene (11) derivative were isolated from *Acalypha macrostachya* (Siems *et al*, 1995).

![Chemical structures](image)

Cycloartane type triterpenes (12, 13) were reported from *Acalypha communis* (Maria Teresa *et al*, 2002).
2.5 Phytochemical constituents of other *Euphorbiaceous* plants

*Euphorbiaceous* plants have afforded many polyfunctional diterpenoids with the tetracyclic tigiliane, ingenane skeletons and the tricyclic daphnane skeleton (Evans, 1978). The diterpene lactone (14) was isolated from *Euphorbia acaulis* (Satti *et al*, 1986).

Pentacyclic diterpene esters (Yan-Ping Shi, 1995) and four diterpene esters (Sevil, 1995) were isolated from *Euphorbia aleppica*.

A large number of triterpenes of lupene type such as lupeol, betulin, lupenone, of friedelane type such as friedelin, friedelanol, friedelan-3, 7-dione, of cycloartane type
such as cycloartenol, its diols and triols and euphol have been isolated from a number of
genera of *Euphorbiaceae* such as *Euphorbia, Phyllanthus* etc. (De Pascual Teresa et al,
1987; Nazli Rasool, 1989; Manuel Fernandes- Ferreira, 1990; Antonio Gonzalez, 1975). Friedelin, betulin and their derivatives were isolated from *Phyllanthus reticulates*. *Euphorbia tirucalli* afforded a taraxerane type triterpene called euphorginol (14a).

Flavonoids are present in many *Euphorbiaceous* plants. Flavonoids such as quercetin, apigenin (Asima Chatterjee, 2003), isovitexin, luteolin glycosides and kaempferol have been reported from many species of *Euphorbia*. Flavanones such as 5,7-dihydroxy-6-methyl-8-prenyl flavanone, and 2',4'-dihydroxy-6'-methoxy-3' prenyl chalcone (Chem Abs, 1984) were reported from *Mallotus philippensis* and flavonoids such as nirunin, a flavone-5-O-rutinoside and 5,3',4'-trihydroxy-flavanone-7-O-α-L(-)-rhamnopyranoside were isolated from *Phyllanthus niruri*. Gallic acid was present in *Phyllanthus fratnernus, Euphorbia hirta* and *Acalypha hispida*.

The compounds isolated from *Acalypha fruticosa* have structural identity as a
class with those isolated from the *Euphorbiaceous* plant *Mallotus philippensis*.

Also no essential oil characterization has been done for any plant of *Acalypha*
genus, though the presence of essential oil was reported for *Acalypha indica* (The Wealth of India, 1988).
2.6 *Aristolochia tagala* Cham—its details, activity studies and therapeutic uses

*Aristolochia* is the largest genus of the family *Aristolochiaceae* which embraces nearly eighteen genera and five hundred species. About 20 species of *Aristolochia* occur in India of which three species namely *Aristolochia bracteolata*, *Aristolochia indica* and *Aristolochia tagala* are useful in medicine. Synonym of *Aristolochia tagala* is *Aristolochia roxburghiana klotzsch*. It is commonly called Oval leaf Dutchmans pipe or India Birthwort.

*Aristolochia* leaves are considered to be tonic, carminative and emmenagogue in the indigenous system of medicine (Angels *et al*, 1970). Its leaves are used as an antidote for snake bite (Henry *et al*, 1994). The anti-implantation and abortificant (Prasad & Vohra, 1959 and Jamual *et al*, 1962) activity of *Aristolochia tagala* has been reported. The roots are frequently used to adulterate *Aristolochia indica* for use in medicine (The Wealth of India, 1985). *Aristolochia indica* was used for treatment of dyspepsia, bowel troubles of children and intermittent fevers. It is prescribed as a tincture and its extracts possess anticancer activity (Kupchan *et al*, 1962).

2.7 Phytoconstituents of *Aristolochia tagala*

Only limited work has been done on this plant. The only available literature regarding the phytoconstituents are the isolation of six crystalline compounds from its roots but, the characterization of only four compounds and the characterization of one compound from its leaf. The compounds isolated from roots were aristolochic acids A and C (15, 16), 7-hydroxyaristolochic acid-A (17) and ailantoin (19) (Ding *et al*, 1981). Leaves of *Aristolochia tagala* afforded 4,7-dimethyl-6-methoxy-1-tetralone (18) (Yu, Zequl, 1984).
2.8 Phytochemical constituents of other *Aristolochia* species

*Aristolochia* species have furnished a variety of classes of natural products like terpenes and terpenoids, alkaloids, lignans and phenyl propanoid derivatives in addition to a variety of rare natural products. The most striking rare compounds which are present invariably in almost all *Aristolochia* species are the aristolochic acids which possess a nitro group in a phenanthrene ring.
Aristolochic acids (20), (21) have been isolated from *Aristolochia indica* (Morris Kupchan *et al.*, 1968), the acids (21) and (24) from *Aristolochia bracteolate* (El, Tahir, 1991; Jagannadha Rao 1958, 1959), the acids and their derivatives (21), (22), (24), (25), (26) and (27) from *Aristolochia longa* (De Pascual Teresa, 1983), the acid (20) from *Aristolochia clementatis* (Gy. Schneider, 1960) and the acids (24), (26) from *Aristolochia indica* (Satyesh Chandra Prakash, 1977). Also *Aristolochia cinnabara* afforded the arislochic acids (20) and (21) (Li Hong, 1994), *Aristolochia brevipis* afforded the acids (20), (24) (Hans Achenbach, 1992) and *Aristolochia argentina* afforded the acids (20, 21, 23) (Horacio *et al.*, 1982).

Aristolactams are also common in *Aristolochia* species. The aristolactams isolated and characterized are aristolactam (28), aristolochic acid- D- methyl ether lactam (29) and aristolactam- β-D-glucoside (30) from *Aristolochia indica* (Morris Kupchan, 1968) and the aristolactams (28,30,32,33,34) were isolated from *Aristolochia kankauensis* (Tian Shung Wu *et al.*, 1993). An acylated N- glycosyl lactam (31) and the lactam (30) were isolated from *Aristolochia contorta* (Lee *et al.*, 1992). Aristolactams (32-35) were isolated from *Aristolochia argentina* (Crohare *et al.*, 1973) and the aristolactam (30) was isolated from *Aristolochia cinnabara* (Li Hong, 1994).
(28) \( R_1 \) \( R_2 \) \( R_3 \)
\( \text{H} \) \( \text{H} \) \( \text{OMe} \)

(29) \( R_1 \) \( R_2 \)
\( \text{H} \) \( \text{OMe} \)
\( \text{OMe} \)

(30) \( R_1 \) \( R_2 \) \( R_3 \)
\( \text{H} \) \( \text{OMe} \)

(31)

(32) \( R_1 \) \( R_2 \) \( R_3 \)
\( \text{OH} \) \( \text{OMe} \) \( \text{H} \)

(33) \( R_1 \) \( R_2 \) \( R_3 \)
\( \text{OH} \) \( \text{OMe} \) \( \text{OMe} \)

(34) \( R_1 \) \( R_2 \) \( R_3 \)
\( \text{OMe} \) \( \text{OMe} \) \( \text{H} \)

(35) \( R_1 \) \( R_2 \) \( R_3 \)
\( \text{OMe} \) \( \text{OMe} \) \( \text{OMe} \)
Some new sesquiterpenes namely ishwarone (36), ishwarane (37), ishwarol (38), aristolochene (39) (Ganguly et al, 1969) were isolated besides 5βH, 7β, 10α- selina-4 (14), 11-diene (40) (Govindachari et al, 1973) from Aristolochia indica. Two isomeric sesquiterpene alcohols (41, 42) (Satyesh et al, 1980) were also isolated from Aristolochia indica.
The steroids 3β-hydroxyl-stigmast-5-en-7-one (43) and 6β-hydroxyl-stigmast-4-en-3-one (44) were also isolated from *Aristolochia indica* (Basudeb Achari et al., 1981) besides aristolindiquinone (45) (Chun-tao et al., 1983).

![Chemical structure of 3β-hydroxyl-stigmast-5-en-7-one (43)](image1)

![Chemical structure of 6β-hydroxyl-stigmast-4-en-3-one (44)](image2)

![Chemical structure of aristolindiquinone (45)](image3)

![Chemical structure of 46)](image4)
Many species of *Aristolochia* have afforded aristolactones. The lactone (46) was isolated from *Aristolochia reticulate* (Martin Smith *et al.*, 1963). Two deoxyribonolactones (47, 48) were isolated from *Aristolochia arcuata* (Mauricio, C. Francisco *et al.*, 2003) and the β-unsaturated-γ-lactone (49) was isolated from *Aristolochia mollissima* (Tian-Shung Wu *et al.*, 2001).

![Chemical structures](image1)

\[ R=H \quad (47) \]
\[ R=H(\text{HPO}_3)_3 \quad (48) \]

Various types of alkaloids have been isolated from different *Aristolochia* species. Tetrahydroisoquinoline alkaloids (50-54) were isolated from *Aristolochia arcuata* (Mauricio *et al.*, 2003) and berberine alkaloids (55, 56) from *Aristolochia consticta* (Luca Rastrelli *et al.*, 1997 and Lucia Moria, 1992).

![Chemical structures](image2)

\[ R=6'-\text{Frc} \quad (50) \]
\[ R=6'-\text{Frc} \quad (51) \]
\[ R=\text{CH}(\text{CH}_2\text{OH})_2 \quad (52) \]
\[ R=\text{CH}_3\text{CH}_3 \quad (53) \]
\[ R=6'-\text{Frc} \quad (54) \]
Besides these, some of the compounds which are commonly found in many *Aristolochia* species are *p*-coumaric acid, allantoin, tyramine derivatives like cis and trans-*N*-feruloyl tyramines, *N*-*p*-coumaryl tyramine, methyl ferulate, methyl-*p*-coumarate, methyl vannilate etc.

Though *Aristolochia* species are characterized by the presence of chemotaxonomic compounds, as seen from the literature, different species also offer different varieties of compounds. Because very little work has been done on *Aristolochia tagala*, its phytochemical investigation was taken up in the present work.

### 2.9 Essential oils from *Aristolochia* species

Some varieties of *Aristolochia* species have afforded essential oils. In majority of the cases, sesquiterpenes are the predominant compounds present. Some of the species which have been investigated for essential oils are *Aristolochia elegans* (Rosen vila *et al*, 1997), *Aristolochia argentina* (Horacio A. Priestap *et al*, 2002), *Aristolochia longa* (De Pascual Teresa *et al*, 1983), *Aristolochia debilis* (Nishida *et al*, 1973), *Aristolochia zenkeri* (Dumont, 1958), *Aristolochia indica* (Krishna Rao, 1935), *Aristolochia asclepiadifolia* (Sagrego *et al*, 1993) etc. In the leaves of *Aristolochia elegans*, sesquiterpene hydrocarbons, in particular β-caryophyllene, isocaryophyllene and bicyclogermacrene were the predominant compounds. In the case of *Aristolochia longa*, the roots gave only the tricyclic sesquiterpenes with aristolane and maaliane skeletons whereas, from the aerial parts, sesquiterpenes were the predominant compounds.
particularly β-caryophyllene and caryophyllene oxide though monoterpenes like linalool and bornyl acetate were also isolated. In the case of *Aristolochia argentina*, the main component present was argentilactone. In all cases, the roots afforded a greater yield of the oil and the composition also varied under the same conditions. The oils were found to possess antifungal and antibacterial activity. There were no reports of the isolation of essential oil from *Aristolochia tagala*.

### 2.10 Chemistry of flavonoids

The flavonoids are one of the most diverse and widespread group of natural products which occupy a prominent position among the natural phenols. The name ‘flavonoid’ is derived from the Greek word ‘flavan’ (yellow). These are important biologically active plant ingredients and about 2% of all carbon photosynthesized is converted into flavonoids (Harborne, J.B., 1988). Flavonoids also known as anthoxanthins are the colouring pigment of plants. The wide range of colours and shades in flowers are principally due to the presence of these colouring pigments in the cells of flower tissues.

Flavonoids occur in a variety of structural forms. All contain a basic C₆-C₃-C₆ fifteen carbon atom structure in their parent nucleus and share the common structural feature of having two phenyl rings linked through a three carbon chain (diphenyl propane derivatives) (Fig-1).
The three carbon chain structure may be transformed into a five or six membered chain through an oxygen atom, present as a substituent on one of the phenyl rings generating a tricyclic system. The tricyclic compound possessing a five membered heterocyclic ring are referred to as auronoids whereas, those possessing a six membered heterocyclic ring are designated as flavonoids.

Natural flavonoids are usually oxygenated and bear hydroxyl and / or methoxyl substituents. A large number of flavonoids occur as O–glycosides in which one or more than one hydroxyl groups are bound to one or more than one sugar moieties through an acid labile hemiacetal bond. In flavonoid C–glycosides, the sugar is C–linked and this linkage is acid resistant. The effect of glycosylation is to render the flavonoid less reactive and more water soluble (Harborne et al, 1975; Harborne et al, 1982).
The characterization of flavonoids involves Shinoda test and colour reactions with sodium hydroxide and ferric chloride. The UV spectrum is very helpful in the identification of flavonoids where two absorption bands are recorded.

<table>
<thead>
<tr>
<th>Band</th>
<th>Wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Band I</td>
<td>300-400</td>
</tr>
<tr>
<td>Band II</td>
<td>240-285</td>
</tr>
</tbody>
</table>

The use of shift reagents like sodium methoxide, sodium acetate and aluminium chloride helps in correlating the position of hydroxyl groups. The mass spectrum of flavonoids shows a characteristic fragmentation due to retro Diels–Alder reactions. The molecular ion \([M]^+\) is usually the base peak for most flavone aglycones. The other major peaks corresponding to \([M-H]^+\), \([M-CO]^+\), \(A^+\), \([A-CO]^+\) are also usually detected.

At present over 2000 flavonoids have been characterized belonging to a number of different classes such as anthocyanins, chalcones, aurones, flavones, flavanols, flavanones, isoflavonoids and bisflavonoids. *Euphorbiaceae* family has produced some flavonoids.

### 2.11 Scope of the work

*Acalypha* is an important genus of the family *Euphorbiaceae*. The Indian variety, *Acalypha indica*, is very popularly used in homeopathic medicines even today for the treatment of a variety of ailments. *Acalypha fruticosa* is often used to adulterate *Acalypha indica*. In view of its importance in homeopathy and since there are no phytochemical reports on this plant, *Acalypha fruticosa* was selected for the present work to explore the presence of newer active constituents. Also, its strong pubescent smell initiated us to work on the essential oil. The extract of the plant showed good antioxidant property.

The second plant chosen was the endangered species *Aristolochia tagala*. All *Aristolochia* species are characterized by aristolochic acids, a nitrophenanthrene derivative (a rare entity), aristolactones and aristolactams. Wide reports on the utility of
*Aristolochia* plants for anticancer and abortificant properties are available. Only a limited study has been done on this plant. So this plant was chosen for the present study. Also, since the other *Aristolochia* species gave a good essential oil, it was planned to isolate and characterize the essential oil and study the antimicrobial activity of *Aristolochia tagala*.

It is hoped that the present study would become a base for further evaluation of these plant species. A systematic work on the isolation and structural elucidation of the different secondary metabolites present in the two selected medicinal plants by spectral methods and the characterization of essential oils from the two chosen plants will form an integral part of the thesis.