

**SUMMARY
&
CONCLUSION**

SUMMARY AND CONCLUSION

SUMMARY.

The traditional systems of medicine like Ayurveda and Siddha derive the drugs mainly from plant source. Due to several reasons plant based systems of medicine are gaining importance all over the world. However, the correct botanical identification of the drug material mentioned in Ayurveda has remained a problem till to-day. A National Pharmacopoeia for the ayurvedic and siddha drugs is not yet available. One of the important problems in ayurveda is the use of more than one botanical source for the same drug; these different botanical sources are claimed to possess similar therapeutic efficacy by the physicians. Such drugs are termed as **Controversial drugs** in ayurveda. Scientific parameters for assessing the identification and therapeutic efficacy of the different sources of such drugs is lacking.

In this thesis, three such drugs viz., Bharangi, Murva and Sariva are investigated for the pharmacognostical, phytochemical and biological activity evaluation.

The different taxa involved are:

1. **Bharangi:**
 - a. *Clerodendrum serratum;*
 - b. *Pygmacopremna herbacea;*
 - c. *Gardenia resinifera.*
2. **Murva:**
 - a. *Marsdenia tenacissima;*
 - b. *Chonemorpha fragrans;*
 - c. *Sansevieria roxburghiana.*

3. Sariva:

- a. *Hemidesmus indicus*;
- b. *Ichnocarpus frutescens*;
- c. *Cryptolepis buchananii*;
- d. *Decalepis hamiltonii*.

The *Ayurvedic Formulary of India* (Anonymous 1978) is considered as the basis for the accepted botanical source of the drug.

The pharmacognostical studies comprises of taxonomic characters of the taxa, macro and microscopic characters, histochemical tests and diagnostic characters of the part used.

The phytochemical studies focus on physical constants like total ash value, acid insoluble ash and water soluble ash, extractive values in alcohol and water, fluorescence analysis, preliminary organic analysis and thin layer chromatography.

The biological activity studies cover antibacterial, anthelmintic, antipyretic, anti-inflammatory and anti convulsant (for species of *Bharangi* only) properties.

1. *Bharangi*.

The accepted source of *Bharangi* is *Clerodendrum serratum* (Anonymous 1978). Taxonomically *Clerodendrum serratum* and *Pygma-copremna herbacea* belong to the same family Verbenaceae whereas *Gardenia resinifera* belongs to Rubiaceae. In habit, *C. serratum* and *G. resinifera* are shrubs or small trees whereas *P. herbacea* is a tiny herb. Leaves are found just above the ground in *P. herbacea* whereas in *C. serratum* and *G. resinifera* they are found on the stem and branches. The flowers are bluish purple in *C. serratum*, pale yellow in *P. herbacea* and white to yellow in *G. resinifera*.

In all the 3 species the root is the part used. It is woody and hollow in *C. serratum*, slender and wiry with globular nodular excrescens in *P. herbacea* while woody and cylindrical in *G. resinifera*. The cork cells are tanniferous in *G. resinifera* whereas tannin is absent in the other two. Stone cells are present in groups in *G. resinifera* while they are found singly in *C. serratum* and *P. herbacea*. The medullary ray cells contain simple starch grains in *G. resinifera*, compound starch grains in *P. herbacea* while they are absent in *C. serratum*.

The different species of the drug Bharangi exhibit considerable variation in the ash values. *G. resinifera* gave the maximum percentage of total ash and water soluble ash while acid insoluble ash was found to be maximum in *C. serratum*. The alcohol and water soluble extractive value percentage were highest in *C. serratum* while lowest in *G. resinifera*. The fluorescence analysis of the powdered drug revealed considerable variation both under visible and ultra violet light.

Preliminary phytochemical analysis revealed the presence of phytosterols, saponins, tannins, glycosides and carbohydrates in all the species. Flavonoids were found only in *P. herbacea* and *G. resinifera*. Gums and mucilages, fixed oils and fats besides volatile oil were totally absent in all the 3 species.

Thin layer chromatography^{NV} revealed 9 spots in *C. serratum*, 8 in *P. herbacea* and 5 in *G. resinifera*. The Rf values varied considerably in all the species.

Antibacterial screening was performed by the cup plate diffusion method to measure the zone of inhibition produced in

comparison to positive control. It was observed that only extracts of *P. herbacea* produced a zone of inhibition and the other two did not show any activity. *P. herbacea* produced a fairly good zone of inhibition on both gram +ve organisms (viz. *Staphylococcus aureus* and *St. citreus*) and gram -ve organisms (*Escherichia coli* and *Pseudomonas aeruginosa*). The minimum inhibitory concentration of *P. herbacea* extract was found to be 250 µgm on all the organisms.

Anti-inflammatory activity using rat paw oedema method was performed. The % inhibition of oedema compared with control was used to assess the anti-inflammatory property. It was found that the positive control phenyl butazone showed 65% inhibition of oedema. In comparison, the extracts of *P. herbacea* and *C. serratum* showed a moderate activity of 56% and 48% inhibition respectively while *G. resinifera* indicated a mild activity of 36% only.

Anti pyretic activity studies were carried out by observing the reduction in temperatures over a period of time when compared to the control after yeast induced pyrexia. It was observed that only the extracts of *G. resinifera* produced a fall in temperature compared to control and positive control paracetamol. The other two species did not show any reduction in pyrexia.

Anti convulsant activity was performed using maximum electro shock method. The extracts were compared on the basis of the reduction in extension period when compared to control. The % of reduction in extension is taken as the parameter for determining the anti convulsant activity. As a routine the time in flexion stage was noted. The recovery of the animal was also observed. The results indicate 92% reduction in extension in the +ve con-

trol of phenobarbitone. In comparison, the extracts of *C. serratum* and *P. herbacea* gave activity of 48% and 45% reduction in extension respectively. *G. resinifera* extract did not show any significant activity.

2. Murva

The species investigated under this drug are: 1. *Marsdenia tenacissima*; 2. *Chonemorpha fragrans* and 3. *Sansevieria roxburghiana*. The accepted source of Murva is *M. tenacissima* (Anonymous 1978).

Taxonomically *Sansevieria roxburghiana* belongs to the monocotyledonous group while *Marsdenia tenacissima* and *Chonemorpha fragrans* are dicotyledons. *S. roxburghiana* is treated under the family Agavaceae while *Chonemorpha fragrans* under Apocynaceae and *Marsdenia tenacissima* under Asclepiadaceae. In their habit, *M. tenacissima* and *C. fragrans* are liana while *S. roxburghiana* is a rhizomatous herb. The leaves are radical in *S. roxburghiana*, simple and opposite in *C. fragrans*, cordate and pubescent in *M. tenacissima*. The flowers are greenish-yellow in *M. tenacissima*, whitish to yellow in *C. fragrans* and greenish-white in *S. roxburghiana*. The fruit is a pair of follicle in *M. tenacissima* and *C. fragrans* while it is capsular in *S. roxburghiana*.

Root is the part used in *M. tenacissima* and *C. fragrans* while it is rhizome in *S. roxburghiana*. In the cork cells of *S. roxburghiana* oil globules are present while they are absent in the other two. The ground tissue contains bundles of raphides in *S. roxburghiana* while in *M. tenacissima*, the cortical tissue con-

tains calcium oxalate crystals and starch grains; it is absent in *C. fragrans*. Medullary rays are uniseriate in *M. tenacissima* and *C. fragrans* while absent in *S. roxburghiana*. The cell contents in *M. tenacissima* consists of calcium oxalate crystals and starch grains, in *C. fragrans* latex tubes and starch grains while in *S. roxburghiana* oil globules, starch grains, acicular crystals are present.

The total ash and the acid insoluble ash percentage was found to be maximum in *C. fragrans* and minimum in *S. roxburghiana*. The water soluble ash percentage was highest in *S. roxburghiana* and least in *C. fragrans*. The alcohol soluble extractive value percentage was highest in *M. tenacissima* and least in *S. roxburghiana*. Likewise water soluble percentage was highest in *M. tenacissima* and least in *C. fragrans*. The powder analysis exhibit considerable variation amongst the species both under visible and ultra violet light.

Phytochemical analysis revealed the presence of alkaloids in *C. fragrans* and *S. roxburghiana*. The steroidal compounds were present in *M. tenacissima* and *S. roxburghiana* while absent in *C. fragrans*. Fixed oils, proteins, saponins, phenolic compounds and carbohydrates were detected in all the 3 species. Gums, mucilage and volatile oils were absent in all the 3 species.

Thin layer chromatogra^{hy} indicated 4 distinct spots in *M. tenacissima* of steroidal nature while the alcoholic extracts gave 3 and 2 spots in *C. fragrans* and *S. roxburghiana* respectively. The spots were all distinguishable from each other.

Antibacterial activity was observed in all the 3 species. However, the results indicate that extracts of *C. fragrans* show a

fairly good activity on both gram +ve and gram -ve organisms. It also exhibited a minimum inhibitory concentration of 125 µg/ml except against *Pseudomonas aeruginosa* when it required a higher concentration of 250 µg/ml to inhibit growth. *S. roxburghiana* extract show a better activity against gram -ve organism *Escherichia coli* compared to the other organisms. It has a MIC value of 125 µg/ml against *E. coli*. *M. tenacissima* showed a milder activity and required a concentration of 250 µg/ml to inhibit growth. Also against *Ps. aeruginosa* it required 500 µg/ml concentration indicating a very weak activity against this organism.

S. roxburghiana exhibited 50% inhibition of oedema on rats induced with paw oedema using carageenan. This is a fairly significant anti-inflammatory activity compared to the positive control phenyl butazone which showed 72% inhibition of oedema.

C. fragrans extract produced 40% inhibition of oedema and *M. tenacissima* extract only 30% inhibition.

The results of antipyretic activity indicate that the extract of *S. roxburghiana* showed a significant activity in comparison to +ve control paracetamol. It not only inhibited an increase in pyrexia compared to the initial pyrexia but brought down the temperature considerably to near normal at the end of 120 minutes. *C. fragrans* did not produce any further increase in pyrexia but also did not show any reduction in pyrexia. *M. tenacissima* did not show any activity.

In vitro screening for anthelmintic activity using earthworms indicate that the extracts of *S. roxburghiana* show a comparatively good activity with piperazine citrate, producing both paralysis and death of the earthworms in 1 minute and 3½

minutes respectively. The activity was moderate in *M. tenacissima* while *C. fragrans* exhibited a very mild activity.

3. Sariva

The taxa *Hemidesmus indicus*, *Ichnocarpus frutescens*, *Cryptolepis buchananii* and *Decalepis hamiltonii* are studied under this drug. The accepted source of Sariva is *Hemidesmus indicus* (Anonymous 1978).

Taxonomically *Hemidesmus indicus*, *Cryptolepis buchananii* and *Decalepis hamiltonii* are under the family Asclepiadaceae (now separated into Periplocaceae) while *Ichnocarpus frutescens* under Apocynaceae. *H. indicus* is a slender twiner whereas the others are stout climbers. In all the four species, the fruit is a follicle.

The root is the part used in all the 4 species. The root of *C. buchananii* is black in colour whereas the other 3 are brownish. A characteristic odour is found in *H. indicus* and *D. hamiltonii* whereas it is absent in the other two. Tannin is present in the cork cells of *H. indicus* and *D. hamiltonii* while absent in the other two. Phloem fibers are present only in *C. buchananii*. Medullary rays are uniseriate in *H. indicus* and *I. frutescens* while it is uni to biseriate in *C. buchananii* and uni to multi-seriate in *D. hamiltonii*. Prismatic calcium oxalate crystals is present in the medullary rays of *C. buchananii* only. Laticiferous cells are present in all the 4 species.

The proximate analysis of the 4 species of Sariva indicate that the total ash value percentage is highest in *D. hamiltonii*, followed by *I. frutescens*, *C. buchananii* and least in *H. indicus*.

The acid insoluble ash percentage is highest in *C. buchananii* followed by *D. hamiltonii*, *I. frutescens* and *H. indicus*. Likewise, the water soluble percentage was found to be maximum in *I. frutescens* followed by *C. buchananii*, *D. hamiltonii* and *H. indicus*. The alcohol soluble and water soluble extractive percentages were found to be maximum in *D. hamiltonii* and least in *I. frutescens*.

The fluorescence analysis of the powders indicated considerable variation in colours.

The phytochemical analysis revealed the presence of phytosterols, saponins, carbohydrates, proteins and phenolic compounds in all the 4 species. Alkaloids were detected only in *I. frutescens* and *C. buchananii*; fixed oils and fats in *H. indicus* and *C. buchananii*; gums and mucilage were absent in all the 4 species.

Thin layer chromatography of petroleum ether extract gave 3 spots in *H. indicus*, 5 in *I. frutescens*, 4 in *C. buchananii* and 5 in *D. hamiltonii* with solvent p. ether:chloroform:benzene (2:1:2) and spraying agent 1:1 vanillic acid; likewise with the system Benzene:Methanol (9.5:5) and 1% vanillic sulphuric acid spraying agent, in petroleum ether extracts, 6 spots were detected in *H. indicus*, 6 in *I. frutescens*, 5 in *C. buchananii*, 6 in *D. hamiltonii*, while in the alcoholic extracts 5 spots each in *H. indicus*, *I. frutescens* and *C. buchananii* whereas 6 in *D. hamiltonii*. The Rf values and colour of the spots indicated considerable variation.

The antibacterial activity was not remarkable in any of the 4 species as evidenced from the zone of inhibition produced.

D. hamiltonii indicated a mild activity on *St. aureus* and *St. citreus* inhibiting growth at 250 µg/ml whereas the activity on *E. coli* is better where the MIC values is 125 µg/ml. *H. indicus* shows a +ve activity on *St. aureus*, *St. citreus* and *E. coli* whereas *I. frutescens* showed a +ve activity only on *St. aureus*. Another distinct observation is that only the extract of *C. buchananii* showed a +ve antibacterial activity on *Ps. aeruginosa* at a MIC value of 250 µg/ml. It also showed a +ve activity on *St. citreus* at the same concentration.

The results of the anti-inflammatory activity indicate that only *C. buchananii* shows a fairly significant activity by inducing a inhibition of oedema of 46.2% compared to 64.8% inhibition shown by +ve control phenyl butazone. This is followed by 35.3% inhibition of oedema produced by *D. hamiltonii* extract. The remaining two plants *I. frutescens* and *H. indicus* showed 26% and 20% of inhibition respectively.

Antipyretic activity screening of the 4 plants indicate that all of them prevented a further increase in pyrexia and also brought down the body temperature when compared to control animals. The comparative results indicate that the maximum reduction in pyrexia was caused by *H. indicus* followed by *I. frutescens*, *C. buchananii* and *D. hamiltonii* respectively.

CONCLUSION.

It is concluded that scientific parameters based on taxonomical, pharmacognostical and phytochemical studies are essential to identify the different botanical sources of the ayurvedic drugs Bharangi, Murva and Sariva. These parameters not only help in

the standardization of these drugs but also in formulating pharmacopoeial standards for ayurvedic drugs.

The exomorphic plant characters of different species of Bharangi, Murva and Sariva have been found to be useful tools to identify the species taxonomically.

It is found that each species exhibit specific exo- and endomorphic characters which help in distinguishing the crude drug (part used) of the respective taxa to the family, genus or at times species level.

The phytochemical analysis data indicate that the different botanical species of the drugs may be identified in powder form on the basis of physical constants, fluorescence and qualitative organic analysis which vary considerably from species to species. Likewise the Rf values of the spots of different species of the drugs differ which help in distinguishing the crude drugs.

It may be concluded on the basis of biological activity studies carried out on the different species of the drug that these taxa may be used and graded as the same ayurvedic drug depending upon the percentage of activity. Thus, in the drug Bharangi, out of the 3 species, only *Pygmacopremna herbacea* is found useful as an antibacterial drug. Anti-inflammatory action though found in all the 3 species, *P. herbacea* exhibited ^{Very Strong} activity followed by *Clerodendrum serratum* whereas *Gardenia resinifera* showed a very mild activity. As an antipyretic drug, only *G. resinifera* responded while anti convulsant activity was intense in *P. herbacea* followed by *C. serratum*.

...the standardization of these drugs...
...for Ayurvedic drugs...
...and characters of different species of plants...
...have been found to be useful tools to...
...the taxonomical...
...is found that each species...
...characters which help in distinguishing...
...of the respective taxa to the family, genus...
...level...
...the botanical...
...social species...
...of the...
...which vary...
...of the...
...help...

⊗

It is further found that the traditional properties of the drugs as mentioned in Ayurveda correspond by and large with the biological activity studies carried out on different botanical sources and thus meet the medical applications mentioned in Ayurveda. The range of percentage of activity in different species helps to monitor the dosage requirements for obtaining the prescribed efficacy of the drug. It is also useful to understand the use or disuse of a specific species for a particular activity.

In the drug Murva, it was found that *Chonemorpha fragrans* and *Sansevieria roxburghiana* are more useful as an antibacterial drug compared to *Marsdenia tenacissima*. Anti-inflammatory activity was more pronounced in *S. roxburghiana* followed by *C. fragrans*. Antipyretic activity was observed only in *S. roxburghiana* while as an anthelmintic drug *S. roxburghiana* may be preferred first followed by *Marsdenia tenacissima* and *Chonemorpha fragrans*.

All the four species of the drug Sariva did not exhibit any antibacterial activity. *Cryptolepis buchananii* gave highly promising anti-inflammatory activity followed by *Decalepis hamiltonii* and *Hemidesmus indicus* while *Ichnocarpus frutescens* may not be useful for this activity. As an antipyretic drug, *H. indicus* will be highly useful while the other species exhibited insignificant activity.

Thus, biological activity studies indicate that though all the botanical species which are claimed to have similar therapeutic efficacy in ayurveda may be used, it may be of importance to identify and prefer species with maximum activity for a particular therapeutic action. (X)

Further, it is also found that the alternate or substitute species in certain cases exhibited a higher percentage of activity than the accepted botanical source of the drug. This factor helps in the optimum utilization of the various taxa and in the conservation and utilization of the germ plasm of medicinal plants in general which is the prime requirement of today for the survival of traditional systems of medicine like ayurveda and siddha.