## CHAPTER 7

### Chapter-7 Summary, Conclusion and Recommendations

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CHAPTER 7

7.1. Summary

The present investigation was an attempt to identify and explore the pharmacological activity of some traditionally used medicinal plants. The plants used for the study are *S. tumbuggaia* Roxb. and *H. ada-Kodien* Schult. These plants were collected and authenticated as per the procedure discussed in the earlier chapters. The dry leaves powder of both the plants was successively extracted with increase in polarity using hot continuous percolation technique. The extracts so obtained were subjected for preliminary phytochemical investigation and physicochemical studies.

7.1.1. Phytochemical investigation

Phytochemical studies proved that the n-hexane extract of both the plants contain steroidal compounds and some resinous substances. Whereas ethyl acetate, methanolic and hydroalcoholic extracts showed the presence of phenolic compounds like flavonoids, triterpenoids and tannins. Methanolic extract of HAKS have shown presence of amino acid.

The ethyl acetate, methanolic and hydroalcoholic extracts of both the plants were subjected for estimation of total phenolic content and flavonoidal content. Among the extracts screened methanolic extracts of both the plants showed highest amount of total phenolic content and flavonoidal content.
7.1.2. *In-vitro* Antioxidant studies

Leaves extracts of both the plants were subjected for the *in-vitro* antioxidant screening on different models were used like DPPH, nitric oxide radical scavenging, hydroxyl radical scavenging, metal chelating ability, superoxide anion radical scavenging, hydrogen peroxide scavenging and reducing power. The procedures and results were discussed in the earlier chapters 5 and 6. From the studies made the methanolic extract of both plant extracts STR and HAKS were shown the potent antioxidant activity and a moderate scavenging activity was observed in HAE-STR and EAE-HAKS when compared with standard compound.

7.1.3. Pharmacological screening for the leaves extracts of STR and HAKS

Based upon the presence of preliminary phytoconstituents, total phenolic, flavonoidal content and *in-vitro* antioxidant studies ME-STR, HAE-STR, EAE-HAKS and ME-HAKS were selected for the *in-vivo* pharmacological screening.

7.1.3.1. Acute toxicity studies

Acute toxicity studies showed that the crude extracts were non-toxic upto a dose of 2000mg/kg and also extended upto 3500 kg/mg (p.o) observed for 0 to 24h, 7 and 14 days. Therefore 2000 mg/kg was
considered as ALD$_{50}$ cut off dose under Globally Harmonised Classification System (GHS) category 5 (safe dose), as per OECD guideline 423 (Annexure 2d).

### 7.1.3.2. Anti-inflammatory activity

Formalin induced anti-inflammatory activity was studied for the crude extracts ME-STR, HAE-STR, EAE-STR and ME-HAKS in a dose ranging from 100, 200 and 400 mg/kg/b.w. The result was evident that the methanolic extract of both STR and HAKS showed highest amount of % inhibition in reduction of paw oedema in dose dependent and time after injection of toxicant formalin (0 to 3h), when compared with control and positive control standard diclofenac sodium (30 mg/kg b.w). The HAE-STR and EAE-HAKS were shown a moderate inhibition in paw oedema respectively.

### 7.1.3.3. Anti-nociceptive activity

Anti-nociceptive activity was evaluated for the crude leaves extracts of ME-STR, HAE-STR, EAE-STR and ME-HAKS by chemical and thermal induced stimuli to induce pain and to study the analgesic effect on both central and peripheral activity on the extracts.

The central acting analgesic activity was observed effective for the methanolic crude leaves extract of both STR and HAKS in the dose ranging from 100, 200 and 400mg/kg b.w. Both the methanolic
extracts were exhibiting potent reduction in the thermally induced pain were compared with the reference standard compound diclofenac sodium as positive control. The HAE-STR and EAE-HAKS were shown a moderate inhibition of pain respectively.

Peripheral acting analgesic activity was performed using acetic acid induced writhing model. The reduction in number of writhing was observed for methanolic extracts of STR and HAKS were showing dose- dependent activity, whereas the HAE-STR and ME-HAKS shown moderate analgesic activity.

Formalin induced pain was studied for both central and peripheral system by observing the early phase (0-5 minute) and later phase (20-35 minute) in terms of paw licking time. The reduction of the paw licking was seen in the early phase due to inhibition of pain by non- inflammatory mediators and later phase shown that the inhibition related to the inflammatory mediators. The methanolic extracts of both the plant showed potent activity at the highest dose 400mg/kg b.w., when compared to the standard reference compound respectively.

7.1.3.4. **Antipyretic activity**

Methanolic crude extract (ME-HAKS) showed potent reduction in brewer’s yeast induced pyrexia. The hyperpyrexia appears to be reducing in the dose-dependent manner for the methanolic extract of
Holostemma ada-Kodien Schult. in the dose of 200 and 400mg/kg b.w with the P value ≤ 0.01, when compared with acetyl salicylic acid (ASA).

7.1.4. Anthelmintic activity screening

Bioassay of anthelmintic activity was performed using Indian adult earthworm (*Pheretima posthuma*), at different concentrations of 50, 100, 200 and 400 µg/ml for the ME-STR, HAE-STR, EAE-HAKS and ME-HAKS leaves extracts respectively. From the observation ME-STR, EAE-HAKS and ME- HAKS extracts showed a potent activity by evaluating the paralytic and death time. The extract showed a similar activity when compared with standard reference compound albendazole respectively.

7.1.5. Isolation and structural elucidation

Column chromatographic separation was performed on ME-STR and Compound I was isolated from ethyl acetate, methanol fraction (76-105) in the ratio of 60:40. The purity was confirmed by HPTLC. The compound was characterized using IR, HNMR and MASS spectral datas and identified as quercetin.

Column separation studies on ME-HAKS were performed to isolate compound II from ethyl acetate, methanol fraction (72-98) in the ratio of 20:80. The purity was confirmed by HPTLC. The compound was characterized using IR, HNMR and MASS spectral datas and identified as gallic acid.
7.2. Conclusion

The conclusions drawn from the results of various investigations performed and discussed in earlier chapters are as follows.

- An ethnobotanical survey was carried out on the traditionally used medicinal plants listed by IUCN red list of threatened species found in southern Eastern Ghats of India. *S. tumbuggaia* Roxb. and *H. ada-Kodien Schult.*, were selected for the investigation based upon the unexplored medicinal use.

- The phytochemical studies concluded that both the plants are rich in flavonoids, tannins, terpenoids, alkaloids and saponin.

- The *in-vitro* antioxidant studies shows that methanolic extracts, ethyl acetate and hydroalcoholic extracts have significant free radical scavenging activity. While comparing the activity with phytochemical screening it was concluded that presence of phenolic compounds and triterpenoids may be responsible for their antioxidant activity.

- Anti-inflammatory and anti-nociceptive activity of various extracts of STR and HAKS was performed and the results proved that methanolic extracts of both the plant extracts have potent activity. Among them ME-HAKS have more potency than ME-STR. This activity was correlated with the total phenolic and flavonoidal contents of both the plant extracts and it was concluded that the presence of more amount
of phenolic and flavonoidal content in ME-HAKS may be responsible for the potency and ME-HAKS showed significant result on the reduction of pyrexia.

- *In-vitro* bioassay of anthelmintic activity on the crude leaves extracts of STR and HAKS showed that the crude methanolic extracts produce a potent anthelmintic activity.

- The chromatographic separation was carried out for the ME-STR and ME-HAKS using gradient elution technique. Compound I was isolated from ME-STR and from spectral studies it was conformed as quercetin. Compound-II was isolated from ME-HAKS and from spectral characterization it was conformed as gallic acid.

### 7.3. Recommendation

The present investigation clearly demonstrates that the methanolic extracts have potent antioxidant, anti-inflammatory, antinociceptive activity, anti-pyretic and anthelmintic activity. The compound isolated from ME-STR was quercetin and the compound isolated from the methanolic extract of ME-HAKS was gallic acid.

Since methanolic extracts were rich in high phenolic compound, alkaloid and triterpenoidal content. Many past literature suggest that these compounds possess all these pharmacological activities performed in this study.
Thus the author recommends that further bioactivity guided isolation on these potent plant extracts may lead to many lead moieties which may serve the human society in curing diseases like rheumatoid arthritis and worm infections.