ABSTRACT

In the present investigation an ethnobotanical studies were carried out on traditionally used folklore medicinal plants belonging to southern Eastern Ghats of India. Kollimalai (Tamil Nadu), Tirupati hills and Nallamalla (Andhra Pradesh) regions were mainly focused to establish the traditional finding on medicinal plants and to explore its analgesic, antipyretic, and anti-inflammatory activity. Two plants selected for the present investigation were *Shorea tumbugaia* Roxb. (Dipterocarpaceae) and *Holostemma ada-Kodien* Schult. (Asclepiadaceae). These plants were found to be mentioned under IUCN Red List of Threatened Species which was used by the indigenous people of southern Eastern Ghats of India.

The selected plants leaves were subjected for the continuous sequential extraction by soxhlation by using solvent in increasing in polarity (n-hexane, ethyl acetate, methanol and hydro-alcohol (1:1)). The extracts were concentrated under reduced pressure to obtain the crude extracts. These crude extracts were used for various studies like physicochemical properties, preliminary phytoconstitution analysis, total phenolic and flavonoidal content, *in-vitro* and *in-vivo* pharmacological studies on antioxidant, anti-inflammatory, anti-nociceptive, antipyretic and anthelmintic activity were performed.
From the preliminary phytoconstituent, *in-vitro* and *in-vivo* pharmacological studies, the most potent extract was selected and subjected for the column chromatography to isolate the phytoconstituent response for the pharmacological activity and characterisation of the isolated compound was studied by using TLC, HPTLC, IR, $^1$HMNR, MASS techniques.

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.

*In-vitro* antioxidant studies of all the extracts were carried out for DPPH, nitric oxide, hydroxyl, superoxide anion, hydrogen peroxide radicals, metal chelating ability and ferric ion reducing power using standard procedures. Among all the antioxidant models used against the leaves extracts of STR and HAKS, the methanolic extracts of both the plants (ME-STR and ME-HAKS) possessed the highest amounts of antioxidant activity followed by HAE-STR and EAE-HAKS.
Acute toxicity studies showed that the crude extracts were non-toxic up to a dose of 2000 mg/kg and also extended up to 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).

Anti-inflammatory studies were evident that the methanolic extract of both STR and HAKS showed highest amount of percentage inhibition after injection of toxicant formalin (0 to 3h). When compared with control and positive control standard diclofenac sodium (30 mg/kg b.w), the percentage inhibition of ME-STR and ME-HAKS at high dose (400mg/kg) was 69.291 ± 3.341% and 77.559 ±2.4787% these values are considered to be significant with P value less than 0.0001 at 3h.

The anti-nociceptive activity was performed for the crude leaves extract of STR and HAKS using various model like chemical induced writhing, formalin induced pain response and hot plate models. The methanolic crude extracts of the both the plant has shown to possess both the central and peripheral analgesic activity.

Methanolic extract of HAKS showed dose dependent antipyretic activity at 200 and 400mg/kg. b.w. with the P value ≤ 0.01, when compared with acetyl salicylic acid (ASA) , but the ethyl acetate extract did to show any significant reduction of pyrexia when compared with reference standard.
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.

Column chromatographic separation was performed for ME-STR and ME-HAKS. The isolated compounds showed the presence of phenolic flavonoids. The purity was confirmed by HPTLC. The compound was further characterized using IR, HNMR and MASS. The spectral datas were used for the identification of isolated compounds. The compounds were identified as quercetin from ME-STR and gallic acid from ME-HAKS. Thus, the results justifies the claim made by the traditional folklore use of the both medicinal plants can be used for the treatment of anti-inflammatory, analgesic, antipyretic and anthelmintic therapy.