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Bioactive Molecules and Pharmacology Studies of *Tephrosia calophylla Bedd.*

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1. INTRODUCTION

Tephrosia calophylla Bedd. is a perennial rhizomatous sub shrub belongs to the family Fabaceae and subfamily Paplilionaceae, *T. calophylla* is commonly known as Adavivempalli, Dumpavempalli, Gaddavempalli and kommuvempalli. It is distributed in the states of Andhra Pradesh, Karnataka, and Tamil Nadu.(Pullaiah et al., 2001; Charyuluet al.,1989). It is mainly available in localities of hillslopes, rare in shady locations. It is found widely in Talakona forest of Andhra Pradesh (Chetty., et al 2008). It is perennial under-shrub, with a height up to 60cm tall with woody root stock, leaves are simple and petiole winged, flowers are in terminal racemes with pink corolla and fruit is pod. *T.calophylla*is used traditionally in folk medicine. According to Ayurveda, the plant is useful as an anti-helminthic, anti-pyretic and as well as an alexiteric drug. It is also active against leprosy, ulcers, cures diseases of the liver, spleen, heart and blood. According to the Unani system of medicine, the root is diuretic, allays thirst, enriches blood, cures diarrhea, it is also useful in bronchitis, inflammations, boils and pimples. Leaves are tonic to intestines. The seeds can be used as substitute for coffee (Kundu and Khare, 1999).

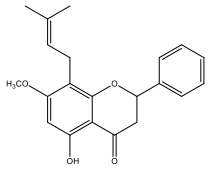
2. BIOACTIVES

The genus *Tephrosia* usually contains a wide variety of flavonoids and isoflavonoids. Investigation on *T. calophylla* revealed that the isolation of 23 different compounds of which 18 were known and 5 are new. A new coumestan, tephcalostan (1) was isolated from the whole plant of *T. calophylla* together with two known flavonoids, 7-O-methylglabranin and kaempferol 3-O-beta-D-glucopyranoside.The structure of tephcalostan was elucidated as 5'-(R)-8, 9-methylenedioxy-5'-isopropenyl-4', 5'-dihydrofurano[2', 3':2, 3] coumestan by extensive one-and two-dimensional (1D- and 2D-)-NMR techniques including (1)H-(1)H correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond connectivity (HMBC) and nuclear Overhauser enhancement spectroscopy (NOESY) experiments (Kishore et al., 2003).Reddy et al. (2009) isolated two new flavanones, (2S)-5-hydroxy-7,4'-di-O-(gamma,gamma-dimethylallyl)flavanone and 6-hydroxy-E-3-(2,5-dimethoxybenzylidine)-2',5'-dimethoxyflavanone together with three known compounds, tephrowatsin C, afrormosin and kaempferol-3-O-beta-D-glucopyranoside from the roots of *T. calophylla*. The structures of two flavones were established by 2D NMR spectral studies. The

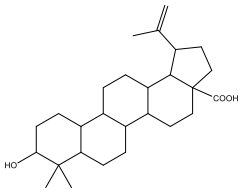
root extracts of *T. calophylla* yielded A benzil, calophione A, 1-(6′-Hydroxy-1′,3′-benzodioxol-5′-yl)-2-(6″-hydroxy-2″-isopropenyl-2″,3″-dihydro-benzofuran-5″-yl)-ethane-1,2-dione and three coumestan derivatives, tephcalostan B, C and D from the roots of *T. calophylla*. Their structures were deduced from spectroscopic data, including 2D NMR ^{1}H-^{1}H COSY and ^{13}C-^{1}H COSY experiments (Ganapaty et al.,2009). Betulinic acid {(3β)-3-Hydroxy-lup-20(29)-en-28-oic acid}was isolated from the whole plant of *T. calophylla* (Subramanyam et al., 2009).

Table 1. Biomolecules from *T. calophylla*

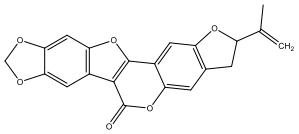
S.No.	Phytoconstituents	Parts used	Identified/isolated Compounds	Reference
1	Coumestan	Whole plant	Tephcalostan,	Kishore et al., (2003)
		Roots	Tephcalostan B, Tephcalostan C, Tephcalostan D	
2	Flavonides	Whole plant	7-O-methylglabranin, Kaempferol (3-O-β-D-glucopyranoside), (2S)-5-hydroxy-7,4'-di-O-(gamma,gamma-dimethylallyl)flavanone, 6-Hydroxy-E-3-(2,5-dimethoxybenzylidine)-2',5'-dimethoxyflavanone, TephrowatsinB, Afrormosin,	Reddy et al., (2009)
3	Benzyl derivative	Roots	Calophione A, (Hydroxy-1′,3′-benzodioxol-5′-yl)-2-(6″-hydroxy-2″-isopropenyl-2″,3″-dihydro benzofuran-5″-yl)-ethane-1,2-dione)	Ganapaty et al., (2009)
4	Acid derivative	Whole plant	Betulinic acid ((3β)-3-Hydroxy-lup-20(29)-en-28-oic acid))	Subramanyam et al., (2009)



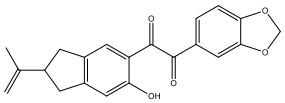
kaempferol 3-O--D-glucopyranoside



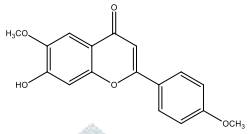
Betulinic acid



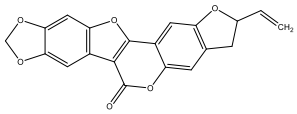
Tephcalostan



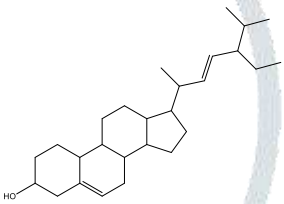
Calophione A



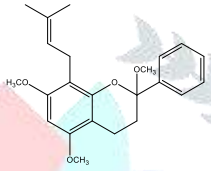
Afrormosin



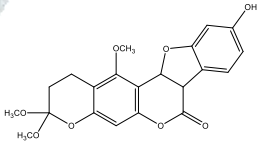
Tephcalostan A



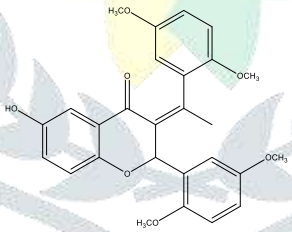
Stigmasterol



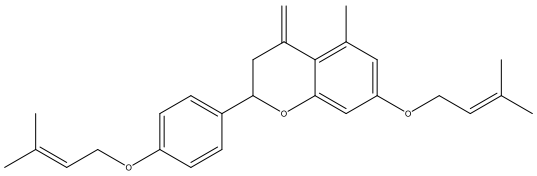
Tephrowastin-C



Tephcalostan C



6-hydroxy-E-3-(2,5-dimethoxybenzylidine)-2',5'-dimethoxyflavanone



(2S)-5-hydroxy-7,4'-di-O-(gamma,gamma-dimethylallyl)flavanone

3. PHARMACOLOGY

3.1 Antidiabetic activity

Ramesh and Rani (2018) reported the anti-diabetic activity of methanolic extract of *T. calophylla* by both *in-vitro* and *in-vivo* methods. Antidiabetic activity against alloxan-induced diabetes in albino wistar rats showed that there was a significant reduction in the blood glucose levels when compared with the diabetic control

group. The extract was also effective in reducing the serum concentrations of serum glutamic oxaloacetic transaminase (SGOT), triglycerides (TG), total cholesterol (TC) and urea, and increased insulin level. *In-vitro* study revealed that the methanolic extract of *T. calophylla* inhibited α-glycosidase and α-amylase activities.

3.2 Antioxidant activity

Yasodamma et al., (2016) evaluated DPPH (2,2-diphenyl-1-picryl hydrazyl) free radical quenching potential of rhizome aqueous extract of *T. calophylla* and copper oxide nanoparticles (CuONPs) atdifferent concentrations (25- 100µg/ml). Ascorbic acid was used as a standard. The findings showed that the extract and the CuONPs exhibited a dose-dependent quenching activity against the DPPH radicals. The increasing order of percentage activity is presented as follows: *T. calophylla* rhizome extract (33.4%) < *T. calophylla* CuONPs (50.2%) < Ascorbic acid (76.6%). It was concluded that CuONPs of *T.calophylla* was found to possess stronger DPPH activity than the rhizome extract alone.

3.3 Antiulcer activity

Divya et al., (2011) investigated the antiulcer activity of ethanolic extract of *T. Calophylla* leaves **(**ETC) in pylorus ligation, ethanol induced and indomethacin induced ulcer methods in wistar rats. Gastric ulcers were induced by oral administration of ethanol, indomethacin and by pyloric ligation. The ETC was administered at a dose of 50 and 100 mg/kg orally. Ranitidine (50 mg/kg) was used as reference standard. In Pylorus ligated rats, oral administration of ETC exhibited significant reduction in ulcer index and prevented formation of lesions which was comparable to that of standard drug ranitidine. In addition, a reduction in volume of gastric contents, total acidity and free acidity was found and the pH was increased significantly by administration of ETC ($p<0.001$) when compared to control group. In the other method, oral administration of absolute ethanol produced characteristic lesions in the glandular portion of rat stomach in control animal. Upon opening the stomach, elongated bands of thick, black and dark red lesions were found in the mucosa. In animals pre-treated with ETC, a significant inhibition of gastric ulceration and reduction in ulcer index was observed ($P<0.001$). In the third method, Indomethacin treatment resulted in the production of gastric lesions, mainly in the glandular segments of the stomach. The ETC produced significant ($P<0.001$) reduction in ulcer index and severity score when compared to control. The pH was also increased significantly by ETC ($P<0.001$) when compared to control group. In a previous study, Adinarayana (2010) *reported* antiulcerogenic activity of *T. calophylla* roots on aspirin plus pyloric ligation induced gastric ulcer model to validate its traditional claim. The results revealed that the ethyl acetate extract of the root powder (ETCR) at the concentrations of 50, 100, 150, 200 and 250 mg/kg, p.o. prevented ulcer formation without any antisecretory effects. The antiulcer activity was further authenticated by the histopathological studies of stomach mucosa. Three coumestan derivatives isolated from ETCR may be responsible for antiulcerogenic activity of *T. calophylla* roots.

3.4 Antimicrobial activity

Ramadevi et al., (2014) reportedantibacterial and antifungal activity of the chloroform extract (100 and 200 mg/ml) of *T. calophylla* root against four gram positive bacteria namely, *Bacillus subtilis*, *Bacillus pumilis*,

Bacillus cereus and *Staphylococcus aureus* and four gram negative bacteria, *Escherichia coli*, *Pseudomonas aeruginosa*, *Pseudomonas vulgaris* and *Serratia marceseans*, and the fungi namely *Aspergillus niger*, *Rhizopus stolonifer*, *Saccharomyces cerevisiae* and *Pencillium chrysogenum* using cup plate method. Chloramphenicol (10 µg/ml) and Nystatin (10µg/ml) were used as reference standards for antibacterial and for antifungal respectively. The activity of the extracts increased with increasing doses. In an another study, Anitha and Sudarsanam (2013) reported antimicrobial activity of hexane, chloroform, ethanol, methanol and water extracts of *T. calophylla* petiole, leaf blade and root against *Xanthomonas citri* and *Salmonella typhimurium*. The findings showed that water extracts of petiole and root displayed highest antimicrobial activity against *X. citri.* Among root and petiole extracts, water extracts showed highest inhibitory activity against *X. citri.* The hexane extract of leaf blade displayed highest inhibitory zone against *X. citri.* Moreover, the water extract of *T. calphylla* leaf blade exhibited the highest antibacterial activity against *S. typhimurium.* In conclusion, it was found that *S. typhimurium* was more susceptible to *T. calophylla*.

3.5 Anthelmintic activity

Devi et al. (2017) studied anthelmintic activity of ethanolic extract (25, 50 and 100 mg/ml) of *T. calophylla* roots and the standard (Albendazole) against adult Indian earthworm, *Pheretima posthuma*, due to its anatomical and physiology resemblance with intestinal round worm's parasite of human beings. The results revealed that the effect of ethanolic extract *T. calophylla* at the concentration of 100 mg/ml displayed significant anthelmintic activity.

3.6 Hepatoprotective activity

Adinarayana et al. (2011) evaluated hepatoprotective activity of *T. calophylla* against CCL_4 induced hepatotoxicity. The animals were pretreated with methanol extract of *T. calophylla* (150 and 300 mg/kg of body weight) for 14 days. It was then treated with CCL_4 (1.5 mL/kg body weight) in olive oil (1:1,v/v) on 14^{th} day. The methanolic extract of *T. calophylla* reduced the elevated levels of SGPT, ALP and bilirubin but not SGOT. The extract of *T. calophylla* treated rats was compared with standard drug Liv.52 treated group and there was no significant difference in biochemical parameters.

3.7 Cytotoxic activity

Ganapaty et al., (2009) evaluated calophione A, tephcalostan B, C and D isolated from *T. calophylla* for cytotoxicity against RAW (mouse macrophage cells) and HT-29 (colon cancer cells) cancer cell lines. Calophione A exhibited significant cytotoxicity with IC_{50} of 5.00 (RAW) and 2.90 µM (HT-29), respectively.

3.8 Anticancer activity

Adinarayana et al., (2009) evaluated root extracts of *T. calophylla* for the cytotoxic activity against human breast carcinoma cell lines. The root extract was found to inhibit the growth and induced apoptosis in human breast carcinoma.

3.9 Antihyperlipidemic activity

Mohan et al., (2011) evaluated the antihyperlipidemic effect of chloroform and methanolic extracts of whole plant of *Tephrosia calophylla* against acute and chronic hyperlipidemia in wistar albino rats by Triton WR-1339 induced model and chloroform-diet induced model. In both models, the serum was analyzed for total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), and using standard methods. Simvastatin (7.2 mg/kg b.w) was used as standard for both models. The chloroform extract (300 mg/kg) and methanolic extract (500 mg/kg) of *T. calophylla* exhibited antihyperlipidemic activity in hyperlipidemic rats. Altered lipid profile parameter was also restored near to normal level. The activity was also supported by the histopathological studies where extracts of whole plant of *T. calophylla* reduced the thickness of aorta.

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