ABSTRACT

The present study aims at the extensive scientific evaluation of methanolic extract of aerial parts of *Avicennia alba* Blume for antifertility activity and some other biological activities (like analgesic, antipyretic, muscle relaxant, antimicrobial and anti oxidant activity) to validate their folklore claim. This is followed by isolation, characterization of isolated compound, GC-MS study and molecular docking study of bioactive compound from extract.

The antifertility activity of the methanolic extract was evaluated by three models such as anti-implantation activity study, estrous cycle study and estrogenic activity study in female rats. In anti-implantation activity, the methanolic extract (100mg, 200mg and 400mg/kg b.w) was administered to female rats from 1 to 7 days of pregnancy and on 10th day laprotomised to count the number of implants, number of corpora lutea and number of resorption sites. In estrous cycle study, the extract (200mg and 400mg/kg b.w) was administered for thirteen days to cover three regular estrous cycles. A vaginal smear from the animals was observed every morning and the duration of each stages of the cycle was noted. In estrogenic activity study, the uterine weight and vaginal cornification were observed. The diameter of uterus, thickness of endometrium and height of the endometrial epithelium were also measured using a calibrated ocular micrometer. The estimation of total protein and cholesterol was carried out using standard method.

The analgesic activity of methanolic extract of *Avicennia alba* Blume aerial parts (100 and 200 mg/kg, p.o.) was evaluated in radiant heat and tail immersion methods. The antipyretic activity was evaluated by brewer's yeast-induced fever models in rats. For muscle relaxant activity study, rotarod was used and rats (weight 25-30 g) were placed on a horizontal wooden rod (32 mm diameter) rotating at a speed 20 rpm. Animals were then placed on the rod and the fall off time was noted. For antibacterial activity study, agar-well diffusion method and determinations of MIC and MBC were carried out. Total phenolic content was expressed as milligrams of Gallic acid equivalent (GAE) per gram of extract and total flavonoid content was
expressed as milligrams of Quercetin equivalent (QE) per gram of the extract. For antioxidant activity study, DPPH radical-scavenging activity, superoxide radical scavenging effect, the Nitric oxide scavenging effect and the hydroxyl radical scavenging effect were done.

The methanolic extract showed significant \((p<0.05)\) 78.47\%, 86.95\% and 97.09\% antifertility activity at the dose of 100mg, 200mg and 400mg/kg respectively. The extract showed significant increase \((p<0.05)\) in duration of diestrous phase and significant decrease \((p<0.05)\) in duration of metaestrous phase as compared to control. The extract at 400 mg/kg body weight showed a significant \((p<0.05)\) increase in uterine weight, diameter of uterus, thickness of endometrium and height of the endometrial epithelium compared to the control. There was increase in vaginal cornification status. The histological examination of the uterus also showed estrogenic influence. A significant increase \((p < 0.05)\) in total protein and cholesterol content in the uterus of standard and test drug treated rats was also observed.

Estrogen activity is responsible for its anti-implantation activity. Presence of phytoestrogens like stigmasterol and phytol etc may be attributed to its female antifertility effect. This is evident from the molecular docking analysis. In the molecular docking analysis, phytol possesses binding energy of -5.2 kcal/mol with the estrogenic receptor (PDB ID- 1DHT). Stigmasterol also possesses binding energy of -7.4 kcal/mol with the estrogenic receptor (PDB ID- 1DHT). This shows the estrogenic activity of phytol and Stigmasterol.

The extract (100 and 200 mg/kg) showed a significant inhibition of elevated body temperature when compared to control and a significant increase in basal reaction time in radiant heat and tail immersion methods. There was significant \((p < 0.05)\) skeletal muscle relaxant activity shown by methanolic extract at 200 mg/kg. The analgesic and antipyretic activity may be attributed to flavonoids present in the plant extract. Muscle relaxant activity of the plant extract might be attributed the presence of Hexadecanoic acid and Octadecatrienoic acid.
The results also revealed that methanolic extract and its various fractions exhibited anti bacterial activity against *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Streptococcus mutans*, *Acinetobacter baumannii*, *Citrobacter freundii*, *Enterobacter aerogenes* and *Escherichia coli*. The methanolic extract as well as its n-hexane fraction had 0.29 mg/ml as the lowest MIC value and 0.67 mg/ml as the lowest MBC value against *Staphylococcus aureus*. It also possesses anthelmintic activity. The anti microbial activity may be attributed to presence of hexadecanoic acid.

The total phenolic and flavonoid content of methanolic exteact of *Avicennia alba* Blume was estimated as 185 mg Gallic acid equivalent/g and 92 mg quercetin equivalent/g respectively. The antioxidant activity of the extract may be attributed to its phenolic and flavonoid content.

The methanolic extract of *Avicennia alba* Blume possesses antifertility, analgesic, antipyretic, antimicrobial and antioxidant activity. This justifies the folklore claims on *Avicennia alba* Blume. A novel flavonoid (2-[3'-(3''-(hydroxymethyl)oxiran-2''-yl)-2'-methoxy-4''-(methoxymethyl)phenyl]-4H-chromen-4-one.) is isolated from the n-hexane fraction of methanolic extract of *Avicennia alba* Blume.