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# GC-MS Profiling and Antifertility Activity of Methanolic Extract of *Avicennia Alba* in Female Rats.

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**ABSTRACT**

The aim of the study was to evaluate the antifertility activity and GC-MS analysis of methanolic extract of *Avicennia alba*. In anti implantation activity study, the methanolic extract (100mg, 200mg and 400mg/kg b.w) was administered to female rats from 1 to 7 days of pregnancy and on 10$^{th}$ day, laprotomised was performed 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. The methanolic extract showed significant ($p$<0.05) 48.94%, 67.36% and 85.76% anti-implantation 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 methanolic extract of *Avicennia alba* possesses antifertility activity due to presence of phytol which justifies the use of this plant for fertility control.

**Keywords:** GC-MS, antifertility activity, estrous cycle, *Avicennia alba,* female rats

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## INTRODUCTION

Fertility control is a dangerous issue for women throughout the world. About 1% of pregnant women loses their lives due to unintended pregnant or obtains an abortion in order to avoid having unwanted child [1]. Since last few decades, most of the research efforts were concentrated tendenciously towards the discovery of oral contraceptives from the synthetic source and no attention was paid to the herbal drugs, although plants are the rich source of bioactive phytoconstituents. Now days, most of the prescriptions for fertility control contain herbal contraceptives. The plants, therefore, provide a broad platform for discovery of new and safe antifertility agents [2]. The synthetic drugs or steroidal contraceptive pills for fertility control show serious adverse effects like gastrointestinal disturbance, depression, hypertension, painful uterine contraction, increased risk of cancer, weight gain and hormonal imbalance [3]. Thus, there is a need of herbal contraceptives agents as they are safe, cheap and may be used with minimum undesirable side effects. The mangrove plant, *Avicennia alba* (Family- Avicenniaceae/Acanthaceae) is available in mangrove forest of saline water in south-east Asia [4]. Fruits and bitter resin of this plant are used as contraceptive agent [5,6] .The plant used as asthma, ulcer, snake-bites, skin disease in traditional system of herbal medicine of india [7,8].

There are folklore claims regarding antifertility activity of aerial parts of plant in the rarrh region of west Bengal. There is no previous report for antifertility activity and GC-MS analysis of *Avicennia Alba*. Hence, we have under taken this study to evaluate the antiimplantation activity with estrous cycle study and GC-MS analysis of methanolic extract of aerial parts of *Avicennia Alba.*

## MATERIALS AND METHODS

### Plant material

The aerial parts (leaves, fruits and stems) of *Avicennia alba,* were collected from Sunderban area, West Bengal, India, in month of October, 2011 and identified by Dr. K. Karthigeyan, Taxonomist, Botanical Survey of India, Howrah, West Bengal. A voucher specimen (CNH/128/2011/TECHII/637 /DRK- 01) has been deposited in the Department of Pharmacognosy, Siksha 'O' AnusandhanUniversity, Odisha for future reference.

### Preparation of plant extract

The aerial parts (1 kg) of *Avicennia alba* were air dried, pulverized to a coarse powder in a mechanical grinder and extracted in a Soxhlet apparatus with methanol (2.5L) for 48 hours. The extract was concentrated to dryness in a Rota evaporator (Buchi type) under reduced pressure and controlled temperature (50-55$^0$C) to yield brown solid mass (16%w/w). The dry extract was preserved in a refrigerator. A suspension of the dry extract was prepared in distilled water using Tween- 80(1% w/v) and used for the present study.

### GC-MS analysis

The GC–MS analysis was carried out using a Clarus 500 Perkin – elmer (Auto system XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perkin Elmer Turbomass 5.1 spectrometer with an Elite – 1 (100% Dimethyl poly siloxane), 30m x 0.25 mm ID x 1µm of capillary column. The instrument was set to an initial temperature of 110$^o$C, and maintained at this temperature for 2 min. At the end of this period the oven temperature was rose up to 280$^0$C, at the rate of an increase of 5$^0$C/min, and maintained for 9 min. Injection port temperature was ensured as 250 $^0$C and Helium flow rate as one ml/min. The ionization voltage was 70eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45-450 (m/z). Using computer searches on a NIST Ver.2.1 MS data library and comparing the spectrum obtained through GC–MS compounds present in the plants sample were identified.

### Identification of components

Interpretation on mass spectrum of GC-MS was carried out using the database of National Institute Standard and Technology (NIST). The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

**Animals**

Wister strain, colony-bred virgin female albino rats (150-200gms) were used. All the animals were provided with rodent diet and water *ad libitum* in animal house. The temperature was 23± $2^0$c and humidity was 50±5%. All animalsexperimental protocol was approved by Institutional Animal Ethics Committee, School of Pharmaceutical Sciences, Siksha 'O' Anusandhan University, Odisha, India. (Registration no-1171/c/08/CPCSEA)

**Acute toxicity study**

OECD guideline 420 was followed for acute toxicity study of methanolic extract of *Avicennia alba* [9].Female, non pregnant rat weighing 150-200 gm were used for this study. The methanolic extract treated animals tolerated the dose up to 4000 mg/kg body weight and there were no death of animals. We use the extract at dose level of 100mg, 200mg and 400mg/kg body weight for present study.

**Anti-implantation activity studies**

Colony bred virgin female albino rats (150-200gm) were used for antiimplantation activity. The vaginal smears from each rat were monitored daily. Only the rats with normal oestrous cycle were selected for the experiment. Antifertility activity was determined as describe by Khanna and Chowdhuary [10]. The female rats were caged with male rats of proven fertility in the ratio of 2:1 in the evening of proestrous and examined the following day for the evidence of copulation. Rats exhibiting the copulation plug or thick clumps of spermatozoa in their vaginal smears were separated and the day was designated as day 1 of pregnancy and those rats were divided into 5 group containg 6 rats in each group. The group(i)received vehicle only (Tween 80, 1%) and acts as control.Group(ii) received ethinylestradiol (Organon India Ltd.,Kolkatta, India) as standard at the dose of 0.45 mg/kg b.w, Group(iii),Group(iv)and Group (v) received methanol extract at doses of 100mg, 200mg and 400mg/kg b.w respectively. the all treatments were given by orally. The above treatments were given from day 1 to 7 of pregnancy and on the day 10 laparotomisedwere performed under light ether anaesthesia using sterile condition. The uteri were examined to determine the number of implantation sites, number of corpora lutea in the ovary and number of resorption sites. The abdominal wound was sutured with sterile sutures in aseptic condition and the rats were allowed to go to term.

The % of anti-implantion and early abortifacient activity were calculated. The summation of antiimplantion and early abortifacient activity gives % of antifertility activity of the tested materials. The calculation formulas are given below [11].

% of anti-implantion activity = 100-(No of implantation/No of corpora luteum) x 100
% abortifacient activity= (No of resorption/No of corpora luteum) x 100
% Total antifertility activity= % of anti-implantion activity +% abortifacient activity

**Estrous cycle study**

The vaginal smear and the duration of estrous cycle of various phases were employed in this study [12,13]. Colony bred female albino rats with normal estrous cycles were selected for the estrous cycle study. A normal estrous cycle in rats normally occurs 4-5 days.the estrous cycle stages are (i) estrous (cornified epithelial cells ),(ii) metaestrous (cornified cells plus leucocytes),(iii) diestrous(leucocytes) and (iv) proestrous (epithelial cells).To study the effect of methanolic extract on the estrouscycle,the above selected animals were divided into three groups contain six animals in each group .The group (i ) received vehicle only (Tween-80,1%) and served as control.Group (ii) and (iii) received methanolic extract at dose of 200mg and 400mg/kg body wt respectively. The treatment was given for thirteen days to cover three regular estrouscycles. A vaginal smear from the experimental animals was observed every morning and the duration of each stages of the cycle noted.

**STATISTICAL ANALYSIS**

Statistical analysis of the differences between the group were analyzed by one-way analysis of variance (ANOVA) followed by Dunnet's t-test. $p<0.05$ was considered as statistically significant. All data are expressed as the mean value ± SD.

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## RESULTS

### GC-MS analysis

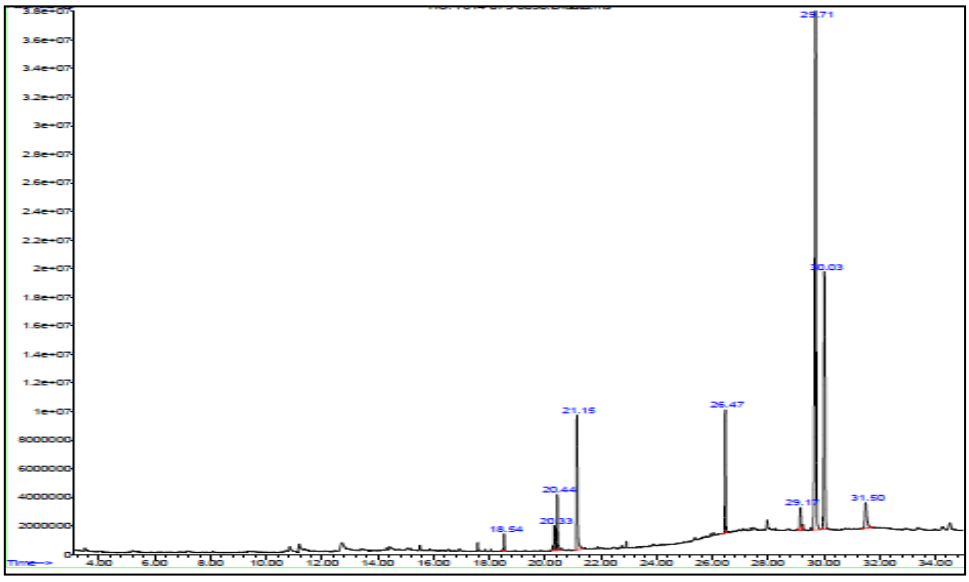
The GC-MS chromatogram of the methanolic extract of *Avicennia alba* (Figure1) showed nine peaks indicating the presence of nine bioactive compounds. The unknown compounds were characterized and identified by comparing their mass spectra with known spectra stored in the NIST-11 library.

These identified compounds with their retention time (RT), molecular formula, molecular weight and peak area (%) chemical nature and activity were depicted in Table-1.

**Table 1: Phytochemical compounds identified in methanolic extract of *Avicennia alba***

| Peak | RT | Name of the compound | Molecular formula | Molecular weight | Peak area (%) | Nature of the compound |
|---|---|---|---|---|---|---|
| 1 | 18.538 | Hexadecanoic acid, methyl ester | $C_{17}H_{34}O_2$ | 270.4 | 0.84 | Palmitic acid |
| 2 | 20.336 | 9, 12, 15-Octadecatrienoic acid, methyl ester,(Z,Z,Z)- | $C_{19}H_{32}O_2$ | 292.4 | 1.20 | Fatty acid ester |
| 3 | 20.440 | Phytol | $C_{20}H_{40}O$ | 296.5 | 3.23 | Acyclic diterpene alcohol |
| 4 | 21.146 | Phenol, 4,4'-(1-methyletyllidene)bis | $C_{15}H_{16}O_2$ | 228.2 | 9.31 | phenolic |
| 5 | 26.472 | Squalene | $C_{30}H_{50}$ | 410.7 | 6.52 | Hydrocarbon, triterpene |
| 6 | 29.169 | Heptanoic acid, anhydrous | $C_7H_{14}O_2$ | 130.1 | 1.83 | Carboxylic acid |
| 7 | 29.704 | Acetamide, 2-cyano-N-(2-phenoxyethyl)- | $C_{11}H_{12}N_2O_2$ | 204.22 | 50.71 | Amide |
| 8 | 30.031 | Cyclopropanecarboxamide, N-(4-fluorophenyl)- | $C_{10}H_{10}FNO$ | 179.1 | 22.52 | Amide |
| 9 | 31.501 | 1,2-Benzisothiazol-3-amine | $C_7H_6N_2S$ | 150.2 | 31.50 | Amino compound |

**Figure 1L: GC-MS chromatogram of methanolic extract of *Avicennia alba***




The results showed that acetamide, 2-cyano-N-(2-phenoxyethyl)-( 50.71%), 1,2-benzisothiazol-3-amine-(31.5%), cyclopropanecarboxamide, N-(4-fluorophenyl)-(22.52%), phenol, 4,4'-(1-methyletyllidene)bis-(9.31%), squalene-(6.52%) and phytol-(3.23%) were found as six major compounds possessing higher concentration in peak area, where as heptanoic acid (1.83%),9, 12, 15-octadecatrienoic acid, methyl ester,(Z,Z,Z)-(1.20%) and hexadecanoic acid, methyl ester (0.84%) were asertained as three minor compounds with lower percentage of peak area in the methanolic extract of *Avicennia alba.*

The three peaks with a maximum area of intensity of 50.71%, 31.5% and 22.52% in the GC-MS analysis correspond to 2-cyano-N-(2-phenoxyethyl)-, 1,2-benzisothiazol-3-amine-and cyclopropanecarboxamide, N-(4-fluorophenyl). The mass spectrum of bioactive compounds such as hexadecanoic acid, methyl ester, 9, 12, 15-octadecatrienoic acid, methyl ester,(Z,Z,Z), squalene and phytol present in the methanolic extract of *Avicennia alba* are presented in Fig. 2A-D.

**Anti-implantation Study**

The result of anti-implantation activity was shown in (Table-2). The study revealed that methanolic extract of *Avicennia alba* at the dose of 100mg, 200mg and 400mg/kg b.w showed significant ($p < 0.05$) antiimplantation activity. The results clearly showed that extract possess antifertility effect in a dose dependent manner. The data showed that dose related responses of antifertility activity produced by extract are more effective compared with control group. The percentage of total antifertility activity showed as 76.35%, 86.94% and 97.08% in the animals treated with 100mg, 200mg and 400mg/kg b.w of methanolic extract respectively. Ethinylestradiol showed antiimplantation activity of 98.34% and served as appositive control.

**Table 2 Post coital antiimplantation activity of aerial parts of *Avicennia alba* of methanol extract (Mean ± SD)**

| Group | Treatment | Dose (mg/kg b.w) | Number of implantation sites | Number of Corpus Leute-um | Number of Resorption | % of Anti-implanta-tion activi-ty | % of Abor-tifacient activity | % of total Antifertility activity |
|---|---|---|---|---|---|---|---|---|
| (i) | Solvent control (Tween 80,1 %) | 1ml/kg b.w | 10.53±1.37 | 11.50±1.37 | 0±0.00 | 8.44% | 0 | 8.44% |
| (ii) | Standard (Ethinylestra-diol) | 0.45 mg/kg b.w | 0.50±0.54* | 10.33±2.25 | 0.33±0.51 | 95.16% | 3.19% | 98.35% |
| (iii) | Methanol extract | 100 mg/kg b.w | 4.16±2.63* | 8.50±1.37* | 2.33±1.36* | 51.06% | 27.41% | 76.47% |
| (iv) | Methanol ex-tract | 200 mg/kg b.w | 2.50±1.22* | 7.66±3.14* | 1.50±0.83* | 67.37% | 19.58% | 86.95% |
| (v) | Methanol ex-tract | 400 mg/kg b.w | 0.83±0.75* | 5.83±1.72* | 0.66±0.51 | 85.77% | 11.32% | 97.09% |

No of animals used each group 6, $*p<0.05$ when compared to control

**Estrous cycle study**

The result of estrouscycle was shown in (Table-3). Treated rats (200mg and 400mg/kg body wt) with methanolic extract of *Avicennia alba* 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. Where as treated rats with different dose level (200mg and 400mg/kg body wt) extract have no significant effect on decreasing in duration of proestrous and increasing estrous phases as compared to control.

**Table 3: The effect of methanol extract of aerial part of *Avicennia alba* on duration of different phases of estrous cycle (Mean duration± SD in days)**

| Group | Treatment | Dose (mg/kg) | Duration of estrous Cycle (days) | Mean days of metestrous | Mean days of pro-estrous | Mean days of estrous | Mean days of diestrous |
|---|---|---|---|---|---|---|---|
| (i) | Control | Tween80 (1ml/kg) | 12.98 ±2.97 | 2.33 ±0.51 | 1.66 ±0.51 | 1.33 ±0.51 | 7.66 ±0.51 |
| (ii) | Methanolic extract | 200mg/kg | 12.99 ±3.38 | 1.66 ±0.51* | 1.50 ±0.54 | 1.50 ±0.54 | 8.33 ±0.51* |
| (iii) | Methanolic extract | 400mg/kg | 12.98 ±3.61 | 1.33 ±0.51* | 1.33 ±0.51 | 1.66 ±0.51 | 8.66 ±0.51* |

No. of animals used each group 6, $* p<0.05$ when compared to control

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Figure 2A: Hexadecanoic acid, methyl ester

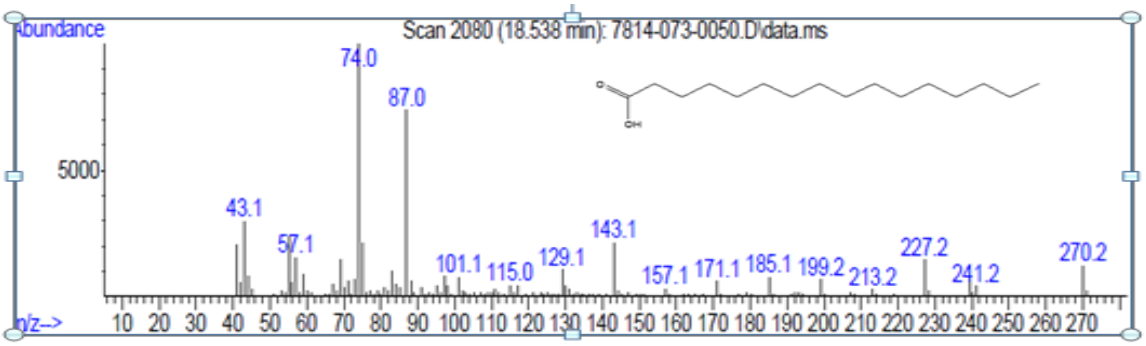


Figure 2B: 9, 12, 15-Octadecatrienoic acid, methyl ester,(Z,Z,Z)-

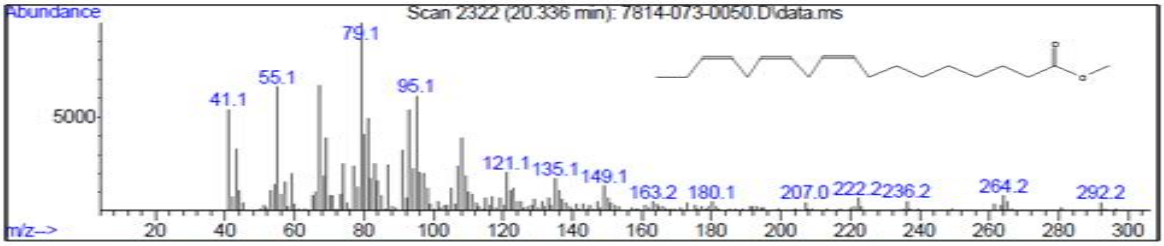


Figure 2C: Squalen

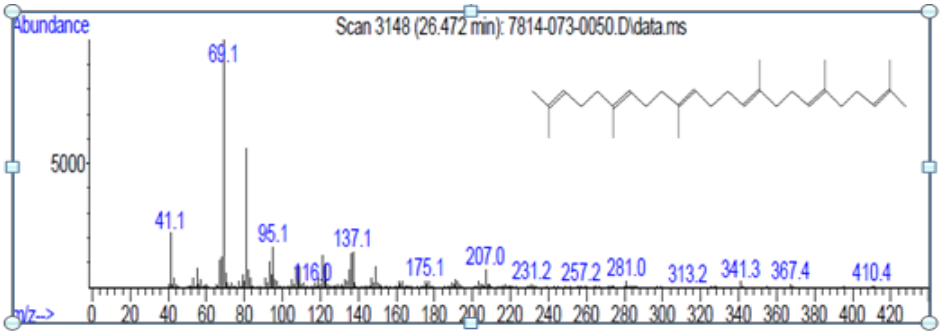


Figure 2D: Phytol

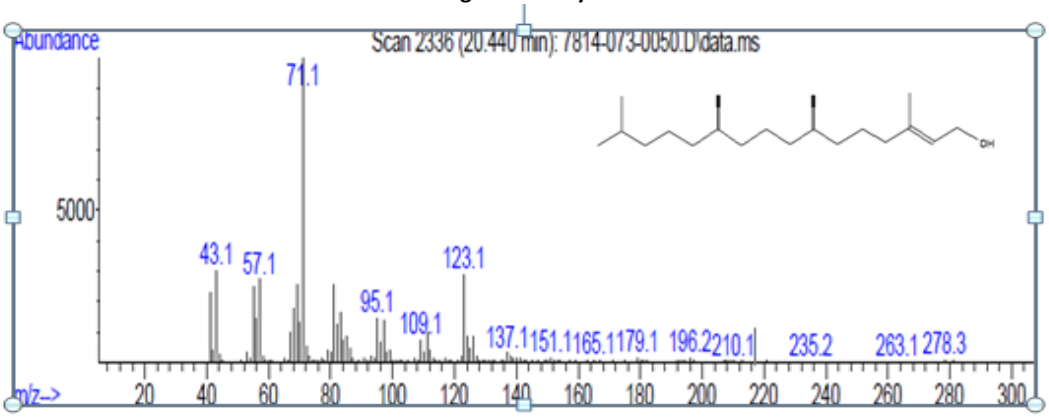

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**Figure 3: Post Coital Anti implantation Activity of Methanolic Extract of Aerial Parts of *Avicennia alba***

**Figure 3A Solvent control**

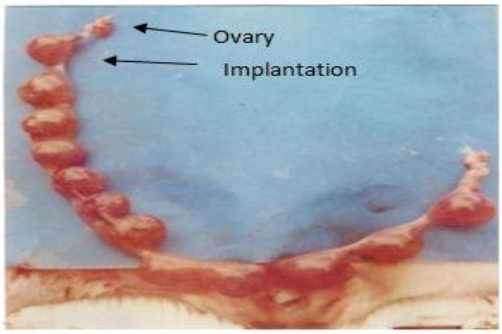



**Figure 3B Stadard drug (Ethinyloestradiol)**

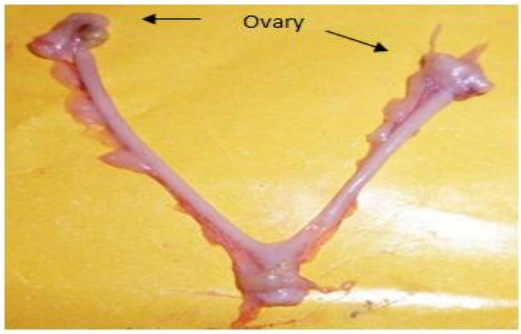



**Figure 3C Methanolic Extract of rial parts(100mg/Kgbody Wt.)**

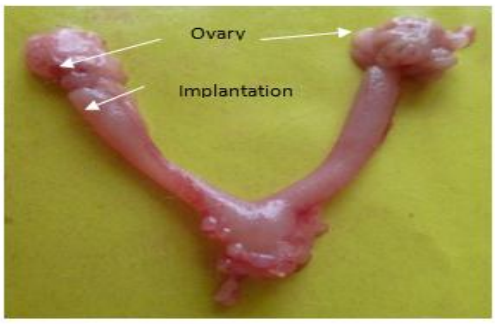
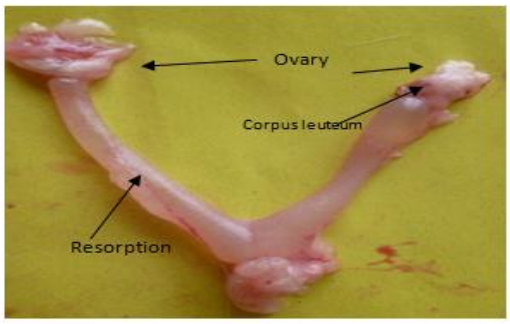



**Figure 3D Methanolic Extract of ae-aerial parts(200mg/Kgbody Wt.)**




## DISCUSSION

To assess the antifertility activity of methanolic extract of aerial parts of *A.alba* in female albino rats by using pharmacological parameters such as antiimplantation activity and estrous cycle study. Implantation is a very crucial event in reproductive physiology. Several biochemical and hormonal changes take place prior to these events in uterus [14]. In the present study, the extract at the dose levels of 100 mg, 200 mg and 400 mg/kg b.wt.showed reduced implantation sites. The loss of number of implantation sites in the uterus may be due to antiimplantation or antizygotic activity [15].

The equilibrium of secretion of female sex hormones like estrogen and progesterone are essential for implantation and maintenance of pregnancy. Any hormonal imbalance can cause antiimplantation or can induce abortion or may cause infertility [16]. Hence, the antiimplantation activity may be due to estrogenic activity causing expulsion of ova from fallopian tube or disruption the luteotrophic activity of the blastocyst [17].

In the present study, methanolic extract of the *Avicennia alba* is analyzed by GC-MS. Till date no reports exist on the GC-MS analysis of this plant extract. The isolated compound, Hexadecanoic acid, methyl ester has earlier been reported for antibacterial, antifungal, Antitumor and anti-inflammatory activity [18,19,20].

9, 12, 15-Octadecatrienoic acid, methyl ester,(Z,Z,Z)- was found to have antimicrobial, analgesic, anesthetic, allergenic, anti-convulsant, anti-inflammatory, antioxidant, antipyretic, anti-salmonella, antiseptic, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, Antieczemic, Antiacne, 5-Alpha reductase inhibitor [21,22].

Squalene was previously reported to have emollient, skin hydration, antitumor activities, antibacterial, antioxidant, immunostimulant, chemo preventive, lipoxygenase inhibitor [23]. The other isolated compounds of this plant extract have not been reported earlier.

The GC-MS analysis of *Avicennia alba* showed the presence of phytosterol like phytol. This phytosterol has been claimed to exhibit estrogenic activity due to its affinity towards estrogenic receptors leading to infertility in animals [24].

The anti-fertility activity of methanolic extract of *Avicennia alba* was observed during the times after implantation process. It was found that, the extract has a dose dependent antiimplantation activity in pregnant rats. The extract also affected the implantation period as illustrated by the decrease in abortifacient activity. Among the tested groups, the group that was treated with the methanolic extract at the dose of 400 mg.kg, b.w exhibited the most potent anti-fertility activity, which is confirmed by the decreasing the number of implantation sites and abortifacient activity. Further, the extract also contained phytol which could be metabolized by hepatic enzyme to phytanic acid after oral ingestion [11].

This compound has also been reported to activate estrogen responsive genes through activating nuclear receptors like peroxisome proliferator-activated receptors (PPARs) and heterodi-merizes with retinoid X receptor (RXR) [25]. Hence, the anti-fertility activity of the methanolic extract of *Avicennia alba* may be due to synergistic action occur in response to its phytosterol and signals emerging from phytol signaling pathways. The present study on the effect of methanolic extract on estrous cycle of female rat shows significant increase in the length of diestrous phase. However a significant decrease in duration of metaestrous phase in the treated groups was recorded than those of control animals. The prolongation of diestrous phase and decrease in metaestrous phase may lower the chances of pregnancy by interfering with secretion of female sex hormone estrogen and progesterone because of a proper estrogen and progesterone balance is required for uterine receptivity to the embryo [26]. This effect may be due to presence of phytoestrogen like phytol that is responsible for disruption of estrous cycle.

## CONCLUSION

In the present study nine chemical constituents have been identified from methanolic extract of the aerial part of *Avicennia alba* by GC-MS analysis. The presence of non steroidal phytoestrogen, phytol justifies the use of this plant for birth control by traditional practitioners. Further work need to be done in future to correlate the other specific compounds with its biological properties.

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