

## Abortifacient and Antioxidant Activities of *Avicennia marina*

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### ABSTRACT

The current study was intended to evaluate abortifacient and antioxidant activity of *Avicennia marina* leaves extracts. Abortifacient activity was evaluated in rats, compared with standard drug (Mifepristone) and antioxidant activity was evaluated by using three free radicals (Superoxide, Hydroxyl and DPPH) compared with Ascorbic acid. The extracts were showed pre implantation loss, post implantation loss of implantations and decreased the survival ration of foetuses. Among all extracts hydroalcoholic extract showed better activity. The selected plant extracts showed concentration dependent percentage inhibition of free radicals. Among three extracts hydroalcoholic extract showed better activity with IC<sub>50</sub> values on superoxide, hydroxyl and DPPH radicals were 203µg, 237µg and 143µg. From the results obtained during the study it the hydroalcoholic extract was used for the isolation of pure compounds, and isolated the different compounds (β-sitosterol, Lupeol and Betulinic acid).

**Keywords:** *Avicennia marina*; Abortifacient activity; Free radicals; Antioxidant Activity

### 1. INTRODUCTION

Medicinal plants will continue to provide a source for generating novel drug compounds. Plants may become the base for the development of a new medicine or they may be used as phyto-medicine for the treatment of disease [1].

Majority of the present day diseases are due to the shift in the balance of the pro-oxidant and the antioxidant homeostatic phenomenon in the body. The antioxidant defense systems can only protect the body when the amount of free radicals are within the normal physiological level; but when this balance is shifted towards more of free radicals, it leads to oxidative stress which may result in tissue injury and subsequent diseases [2].

Abortifacient agents obtained from indigenous medicinal plants would be of immense benefit especially to inhabitants of developing countries, since the cost of these drugs would be within their reach. The antifertility plants with estrogenic property can directly influence pituitary action through peripheral modulation of luteinizing (LH) and follicle-stimulating hormones (FSH) by decreasing the secretion of these hormones and blocking ovulation. In

addition, the plants may also intercept the synchronized development of the ovum and endometrium while others may have abortifacient or antiprogestational effects. In different parts of the world, herbal substances have been used in abortion for unwanted pregnancy conditions and to restrict human population by exploring their abortifacient properties because of the use of synthetic drugs leads to vigorous systemic illness or even death [3-6]. In recent days many researchers reported the folklore claims of abortifacient plant used by the people scientifically [7-12].

On ethnobotanical survey on different medicinal plants which are used by the tribal people in araku valley region of Visakhapatnam District, Andhra Pradesh, India claimed to be used to abortion was *Avicennia marina* [13]. There is no information in the open scientific literature that has substantiated or refuted the abortifacient claim of this. Therefore, the aim of this study was to evaluate the abortifacient activity of different extracts of *A. marina* with a view to validating their acclaimed use by the tribal people of Araku.

## 2. MATERIALS AND METHODS

### 2.1. Collection of plant material and preparation of extracts

The plant material was collected at Araku valley, Visakhapatnam district, Andhra Pradesh, India and the plant was authenticated by taxonomist Prof. M. Venkaiah, Department of Botany, Andhra University. The collected plant material was shade dried and pulverized into powder. The powdered material was used for extraction with different solvents (Hexane, Ethyl acetate, and Hydro alcoholic) using maceration process. Then the extracts were used for screening antioxidant and abortifacient activities.

### 2.2. Selection of Animals

Wistar albino rats of weighing between 150-200 g were obtained from National Institute of Nutrition, Hyderabad, Andhra Pradesh, India. The animals were housed under standard environmental conditions (temperature of  $25 \pm 2^{\circ}\text{C}$  with an alternating 12h light-dark cycle and relative humidity of  $50 \pm 15\%$ ), one week before the start and also during the experiment as per the rules and regulations of the Institutional Animal Ethics Committee and of the regulatory body of the government (Regd no. 516/01/A/CPCSEA). They were fed with standard laboratory diet during the experiment.

### 2.3. Chemicals

Test drugs	: Different extracts of <i>A. marina</i> leaves (at 125 and 250 mg/kg b.w doses)
Standard drug	: Mifepristone (2.85 mg/Kg b.w)
Drug vehicle	: 1% Gum acacia

1,1- diphenyl-2-picrylhydrazyl was purchased from Sigma chemicals, USA. Nitroblue tetrazolium was purchased from Sisco Research Laboratories Pvt Ltd., Mumbai. Riboflavin was purchased from Loba Chemie Pvt Ltd., Bombay.

## 2.4. Acute toxicity studies

Acute toxicity study was conducted according OECD Guide lines No.423. After fasting overnight, mice were administered with extracts of *A. marina* in a single dose up to the highest dose of 2000 mg/kg orally. The animals were observed continuously for 1 h and then hourly for 6 h and finally after every 24 h up to 15 days for any toxicological symptoms or mortality.

## 2.5. Qualitative and Quantitative Phytochemical Screening [14, 15]

Qualitative phytochemical screening was carried out using different standard phytochemical tests for different compounds. Quantitative estimation of phenols and alkaloids were carried out using Folin-Ciocalteu reagent and Bromocresol Green solution. The total phenols were expressed in phenol content in Gallic acid total equivalents and total alkaloid content were expressed in Atropine total equivalents using unit's mg/gm

## 2.6. *In Vitro* anti oxidant activity [16-18]

For the assessment of free radicals scavenging activity, hexane, ethyl acetate, Ethanol (70%v/v) and methanol extracts were dissolved in dimethyl sulphoxide (DMSO) respectively.

### 2.6.1. Superoxide radical Scavenging activity

Superoxide scavenging activity of the plant extract was determined by McCord & Fridovich method, which depends on light induced superoxide generation by riboflavin and the corresponding reduction of nitroblue tetrazolium [17].

### 2.6.2. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity is commonly used to evaluate the free radical scavenging effectiveness of various antioxidant substances [19]. Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated from the  $\text{Fe}^{2+}/\text{EDTA}/\text{H}_2\text{O}_2$  system (Fenton reaction). The hydroxyl radical attacks deoxyribose, which eventually results in the formation of thiobarbituric acid reacting substances (TBARS).

### 2.6.3. DPPH radical Scavenging activity

The scavenging activity for DPPH free radicals was measured according to the procedure described by Braca [20]. In DPPH assay method is based on the reduction of alcoholic DPPH solution (dark blue in colour) in the presence of a hydrogen donating antioxidant converted to the non radical form of yellow colored diphenyl-picrylhydrazine. Lower the absorbance higher the free radical scavenging activity [21].

## 2.7. Abortifacient activity [7, 22-24]

Rats exhibiting three consecutive regular estrus cycles were chosen for the study. The female rats in proestrus phase were mated with male rats of known fertility in the ratio of 2:1

in the evening. Female rats exhibiting thick clumps of spermatozoa in the vaginal smear were chosen for the study and that day was considered as day one of pregnancy. The pregnant rats were divided into twenty groups of six each. Group I served as a control, which received 2mL of 1% gum acacia (p.o. daily). Groups II received standard drug (Mifepristone) and groups III to VIII received different extracts of *A. marina* leaves (at 250 and 500 mg/kg b.w doses) (p.o. daily) respectively, six females with vaginal plug/sperm-positive rats will be sacrificed on gestational day 6 for determination of number of corpora lutea and implantations. On 18<sup>th</sup> day of pregnancy six rats were laparotomised under light ether anesthesia. The number of implantation sites and live fetuses were noted in both horns of the uterus. The observations of the drug-treated groups were compared with control group. The following parameters will be observed.

- Number of corpora lutea
- Number of implantations
- Implantation index =  $\frac{\text{Total number of implantation sites}}{\text{Number of corpora lutea}} \times 100$
- Number of live foetuses
- Number of dead foetuses
- Survival ratio =  $\frac{\text{Number of live foetuses}}{\text{Number of live foetuses} + \text{Number of dead foetuses}} \times 100$
- Preimplantation loss =  $\frac{\text{Number of corpora lutea} - \text{Number of implantations}}{\text{Number of corpora lutea}} \times 100$
- Postimplantation loss =  $\frac{\text{Number of implantations} - \text{number of live fetuses}}{\text{Number of implantations}} \times 100$

### 3. RESULTS

#### 3.1. Qualitative and Quantitative phytochemical screening of *A. marina*

Qualitative phytochemical screening of *A. marina* extracts revealed the presence of different phytochemical constituents like steroids, terpenoids, flavonoids, alkaloids, glycosides, phenols, tannins and carbohydrates. The extracts gave negative results for the amino acids, oils and saponins, but all extracts revealed the presence of steroids, terpenoids, phenols, alkaloids, carbohydrates and glycosides and gave negative results to amino acids, saponins, quinines and oils. The hydroalcoholic and ethyl acetate extracts revealed the presence of flavonoids and tannins but the hexane extract gave negative results. The results were showed in Table 1.

The Quantified phenolic contents of *A. marina* extracts were ranging from 12.82±2.62 to 36.42±1.46 (mg/gm). The hydroalcoholic extract have more phenolic content i.e. 36.42±1.46 (mg/gm) than other extracts. The quantified alkaloid content was ranging from 11.44±3.28 to 30.02±2.12 (mg/gm). The hydroalcoholic extract has more alkaloid content i.e. 30.02±2.12 (mg/gm) than other extracts. The results were shown in Table 2.

**Table 1.** Phytochemical constituents in different extracts of *A. marina* leaves.

Phytochemical constituents	<i>Avicennia marina</i>		
	Hexane extract	Ethyl acetate extract	Hydro alcoholic extract
Phytosterols	+	+	++
Terpenoids	+	++	+
Glycosides	+	+	++
Saponins	-	-	-
Flavonoids	-	+	+
Tannins	-	+	+
Carbohydrates	+	+	++
Alkaloids	+	+	++
Amino acids	-	-	-
Oils	-	-	-
Phenols	+	+	++

+, ++ = Present, - = Absent

**Table 2.** Total phenolic and alkaloid content (mg/gm) of *A. marina* leaves.

Name of the extract	Total Phenolic content (mg/gm)	Total alkaloid content (mg/gm)
Hexane	12.82±2.62	11.44±3.28
Ethyl acetate	24.68±1.22	22.38±1.66
Hydro alcoholic (Ethanol 70%)	36.42±1.46	30.02±2.12

### 3.2. Isolation of different compounds from Hydroalcoholic extract of *A. marina* leaves

The hydroalcoholic extract showed the more effective activity (Abortifacient and Antioxidant) compared to Ethyl acetate and Hexane extracts and also the same extract was used for separation of compounds using column chromatography. Three compounds were isolated during the different fractional collections and they are named as AMS-1, AMS-2 and AMS-3.

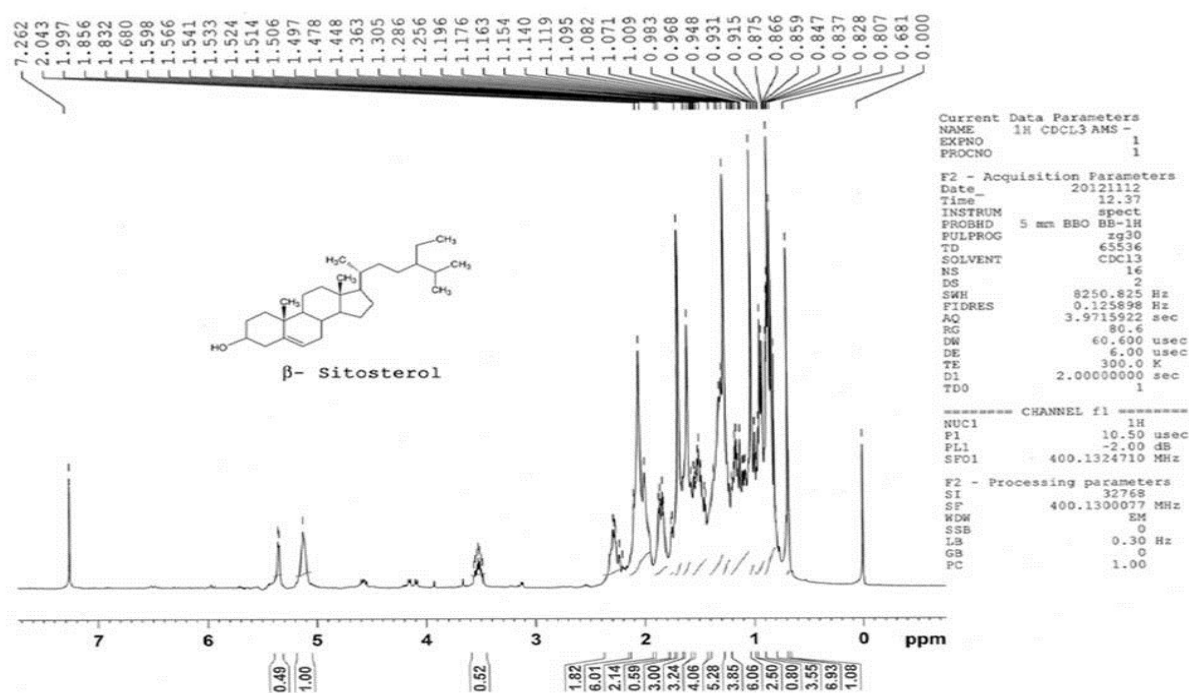
#### 3.2.1. Structure elucidation of the compound AMS-1

The compound was crystallized from 95% Hexane and 5% Ethyl acetate as colourless fine needles, m.p 135-140°C. It showed positive colour reaction with L.B test for steroids, and analyzed for the formula  $C_{36}H_{54}O_2$ .

The  $^1H$  NMR spectrum showed peaks at 0.80 – 1.25 (methyl's), 3.50 (1H,  $3\alpha$ -H), and 5.30 (1 H, m, C5 -H) (Table 3 and Fig 1). Based on the above data the compound was identified as  $\beta$ -sitosterol. It was further confirmed by comparison with authentic sample through Co-TLC and melting points.

**Table 3.**  $^1\text{H}$  NMR spectral data of compound AMS-1 ( $\beta$ -sitosterol).

Assignment	$^1\text{H}$ NMR $\delta$ ppm (300 MHz, $\text{CDCl}_3$ )
methyl's	0.80-1.25
3-H	3.5 (1H, m)
H-5	5.2 (1H, m)
O-H	5.0 (1H, m)

**Fig. 1.**  $^1\text{H}$  NMR spectral figure of  $\beta$ -sitosterol.

### 3.2.2. Structure elucidation of the compound AMS 2

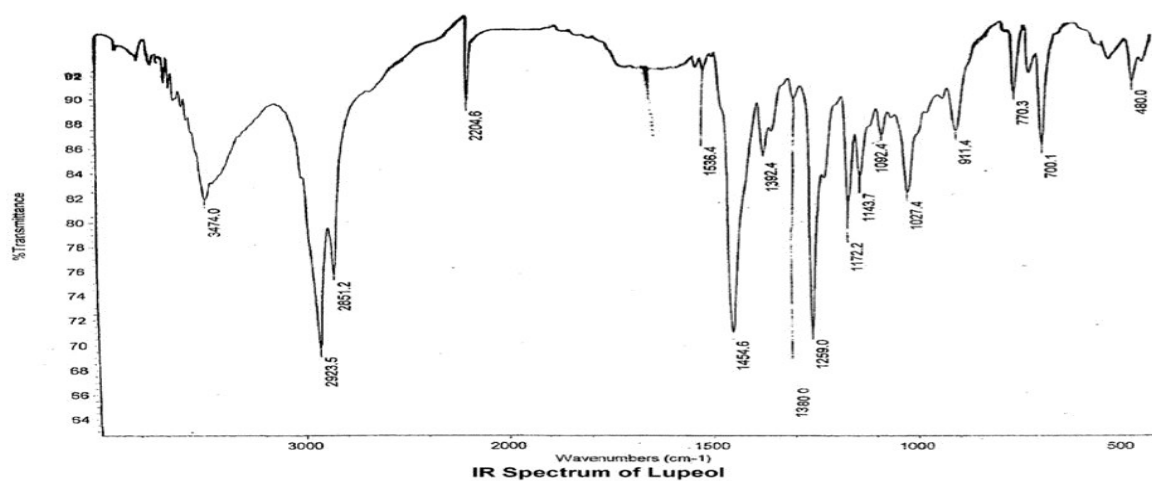
The compound was crystallized from hexane and ethyl acetate as colorless crystals, m.p 195-196°C and analyzed for the formula  $\text{C}_{30}\text{H}_{50}\text{O}$  by mass spectral data and elemental analysis. It gave positive L.B test for triterpenes and yellow colour with tetranitromethane. The IR spectrum showed bands at 3540 (-OH), 1380 and 1390 (gem dimethyl) and 890  $\text{cm}^{-1}$  (vinyl methylene).

$^1\text{H}$  NMR spectrum (300 MHz,  $\text{CDCl}_3$ ) exhibited signals at 0.787, 0.80, 0.828, 0.909 and 1.029 (18H, s, 6  $\text{CH}_3$ ), 1.63 (3H, s,  $\text{CH}_3\text{-C=CH-}$ ), 2.25 (1H, d, 19-H), 3.15 (1H, m, 3 $\alpha$ -H), 4.51 (2H, d,  $\text{CH}_2$ ). The  $^{13}\text{C}$  NMR data (Table 4, Fig 2 and Fig 3) showed chemical shift value of  $\delta$  150.9 ppm for  $\text{C}_{20}$  which is characteristic of lupine derivatives. It has also showed chemical shift value of  $\delta$  78.9 ppm for  $\text{C}_3$  which is characteristic of presence of 3 $\beta$ -OH group.

The  $^{13}\text{C}$  NMR spectral data (300 MHz,  $\text{CDCl}_3$ ) was in good agreement with that of lupeol and the identity was confirmed by comparison with an authentic sample of Lupeol (mixed m.p and Co-TLC).

**Table 4.**  $^{13}\text{C}$  NMR spectral data of compound AMS-2 (Lupeol).

Position	$^{13}\text{C}$ ( $\delta$ ppm, $\text{CDCl}_3$ , 75 MHz)	position	$^{13}\text{C}$ ( $\delta$ ppm, $\text{CDCl}_3$ , 75 MHz)
1	38.7	16	35.6
2	27.4	17	43.0
3	79.0	18	48.3
4	38.7	19	48.0
5	55.3	20	150.9
6	18.3	21	29.8
7	34.3	22	40.0
8	40.8	23	28.0
9	50.4	24	15.3
10	37.1	25	16.1
11	20.9	26	16.0
12	25.1	27	14.5
13	38	28	18.0
14	42.8	29	109.3
15	27.4	30	19.3

**Fig. 2.** IR Spectrum of Lupeol.

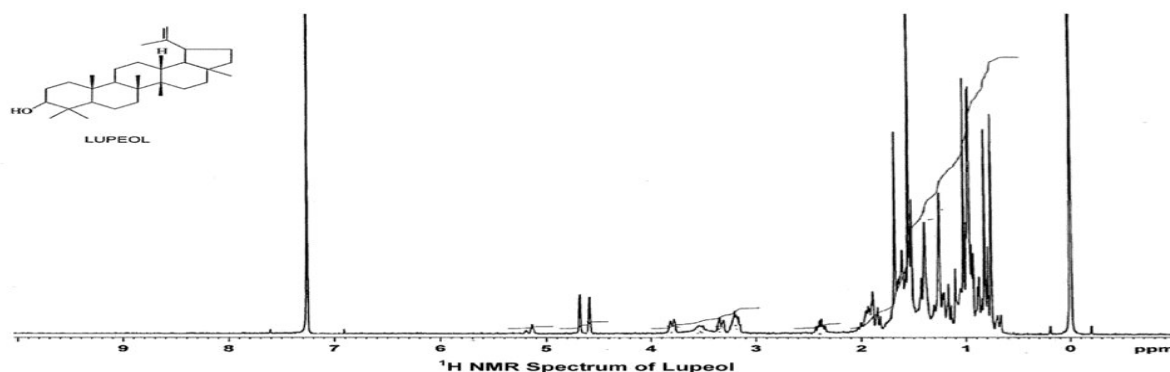


Fig. 3.  $^1\text{H}$  NMR spectrum of Lupeol.

### 3.2.3. Structure elucidation of the compound AMS-3

The compound was crystallized from ethyl acetate - methanol as shining silky needles, m.p  $294-296^\circ\text{C}$ ,  $[\alpha]_D^{30} + 8.6^\circ$  ( $\text{CHCl}_3$ ,  $C=0.5$ ) and analyzed for the formula  $\text{C}_{30}\text{H}_{48}\text{O}_3$  by mass spectral data and elemental analysis. It gave pink colour in L.B. reaction. The IR spectrum showed absorptions at  $3460$  ( $-\text{OH}$ ),  $1690$  (carbonyl of  $\text{COOH}$ ),  $1640$  (double bond),  $1380$  and  $1390\text{ cm}^{-1}$  (gemdimethyl). It formed a mono acetate, m.p.  $287-290^\circ\text{C}$ ,  $[\alpha]_D^{30} + 12.2$  ( $\text{CHCl}_3$ ,  $C=0.5$ ) with  $\text{AC}_2\text{O}/\text{P}_Y$ , a monoester with diazomethane, m.p  $220-221^\circ\text{C}$ ,  $[\alpha]_D^{30} + 8.5^\circ$  ( $\text{CHCl}_3$ ,  $C=0.5$ ), a methyl ester acetate with  $\text{AC}_2\text{O}/\text{Pyridine}$ , m.p  $195-198^\circ\text{C}$   $[\alpha]_D^{30} + 14.2^\circ$  ( $\text{CHCl}_3$ ,  $C=0.5$ ). With the  $^1\text{H}$  NMR data of the compound was identified as betulinic acid (Fig 4).

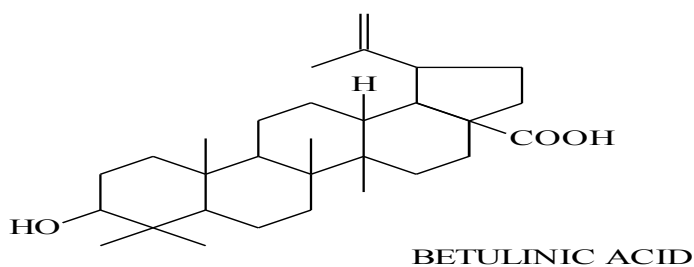


Fig. 4. Betulinic acid.

### 3.3. Abortifacient activity of different extracts of *A. marina* and isolated compound (AMS-2)

The reduction in number of live fetuses shown by administration of 250 and 500 mg/kg b.w of the different extracts of *A. marina* varies in between 13.79% to 40.42%



(survival ratio), while no live fetus was observed with standard drug. The hydroalcoholic extract at a dose of 500mg/kg b.w has shown significantly decreased the survival of foetuses i.e. 13.79%, the preimplantation and postimplantation loss were 54.69% and 86.21% respectively.

The implantation index of all extracts was not more different from each other in all the treated groups but in hydroalcoholic extract at a dose of 500mg/kg b.w show significant i.e, 45.31%, but the percentage of survival ratio and postimplantation loss was varies. The postimplantation loss was more significant than preimplantation loss at both doses of three extracts (Hydroalcoholic, Ethyl Acetate and Hexane). All the extracts of *A. marina* more significantly decreased the number of live foetuses in all treated groups. The results were showed in Table 5.

The order of survival ratio of all extracts was

Mifepristone (0%) < *A m* Hyd. alc Ext 500mg/kg b.w (13.79%) < *A m* Hyd. alc Ext 250mg/kg b.w (24.39%) < *A m* E. A Ext 500mg/kg b.w (25.64%) < *A m* Hex. Ext 500 mg/kg b.w (31.71%) < *A m* E. A Ext 250mg/kg b.w (36.59%) < *A m* Hex. Ext 250 mg/kg b.w (40.42%) < Control (100%).

The order of preimplantation loss of all extracts was

Mifepristone (90.91%) > *A m* Hyd. alc Ext 500mg/kg b.w (54.69%) > *A m* E. A Ext 500mg/kg b.w (35.00%) > *A m* Hyd. alc Ext 250 mg/kg b.w (31.67%) > *A m* E. A Ext 250 mg/kg b.w (29.31%) > *A m* Hex. Ext 500mg/kg b.w (28.07%) > *A m* Hex. Ext 250 mg/kg b.w (24.19%).> Control (15.38%).

The order of postimplantation loss was

Mifepristone (100%) > *A m* Hyd. alc Ext 500 mg/kg b.w (86.21%) > *A m* Hyd. alc Ext 250 mg/kg b.w (75.61%) > *A m* E. A Ext 500 mg/kg b.w (74.36%) > *A m* Hex. Ext 500 mg/kg b.w (68.29%) > *A m* E. A Ext 250mg/kg b.w (63.41%) > *A m* Hex. Ext 250 mg/kg b.w (55.57%).> Control (0%).

The order of abortifacient activity of different extracts of on the basis of postimplantation loss and survival ratio

*A.m* Hyd. alc Ext 500 mg/kg b.w > *A.m* E. A Ext 500 mg/kg b.w > *A.m* Hex. Ext 500 mg/kg b.w.>Lupeol at 100mg/K.g b.w.

**Table 5.** Abortifacient activity of different extracts of *A. marina* at 250 mg/kg b.w and 500 mg/kg b.w and isolated compound AMS-2 (Lupeol) at 100mg/Kg b.w.

S.no	Parameter	Control	Standard (Mifepristone)	<i>A m</i> Hyd. alc Ext		<i>A m</i> E. A Ext		<i>A m</i> Hex. Ext		AMS-2
				250 mg/kg b.w	500 mg/kg b.w	250 mg/kg b.w	500 mg/kg b.w	250 mg/kg b.w	500 mg/kg b.w	100 mg/kg b.w
1.	Number of corpora lutea	8.66 ± 0.36	9.17 ± 0.29	10 ± 0.24	10.67 ± 0.20	9.8 ± 0.20	10 ± 0.35	10.33 ± 0.25	9.50 ± 0.31	10.7 ± 1.21
2.	Number of implantations	7.33 ± 0.23	0.83 ± 0.16	6.83 ± 0.25	4.83 ± 0.13	6.83 ± 0.22	6.50 ± 0.27	7.83 ± 0.25	6.83 ± 0.27	9.3 ± 0.52
3.	Implantation index (%)	84.62	9.09	68.33	45.31	70.7	65.0	75.8	71.9	87.44
4.	Number of live foetuses	7.33 ± 0.23	0.00	1.67 ± 0.09	0.67 ± 0.09	2.5 ± 0.09	1.67 ± 0.20	3.17 ± 0.16	2.17 ± 0.13	8.33 ± 0.82

5.	Number of dead fetuses	0.00	0.83 ± 0.16	5.17 ± 0.20	4.17 ± 0.07	4.33 ± 0.14	4.83 ± 0.16	4.67 ± 0.09	4.67 ± 0.17	1.0 ± 0.63
6.	Preimplantation loss (%)	15.38	90.9	31.67	54.69	29.3	35.0	24.2	28.1	12.56
7.	Postimplantation loss (%)	0.0	100	75.6	86.2	63.4	74.4	55.5	68.3	10.72
8.	Survival ratio (%)	100	0.00	24.39	13.79	36.6	25.5	40.3	31.7	89.28

### 3.4. Antioxidant activity of *A. marina*

The selected plant extracts were produced concentration dependent percentage inhibition on tested free radicals, among the three extracts of *A. marina* the hydro alcoholic extract showed better activity than remaining extracts and the extracts showed more activity on DPPH free radical and equal activity on Hydroxyl and superoxide free radicals. The results were shown in Table 6 and Fig 5.

The mean IC<sub>50</sub> values for superoxide radical of hydroalcoholic, ethyl acetate and hexane extracts of *A. marina* were found to be 203µg, 279µg and 349µg respectively. The mean IC<sub>50</sub> value of ascorbic acid was found to be 59µg. Among all extracts hydroalcoholic extract at a concentration of 640µg showed the better scavenging activity on superoxide free radical i.e. 64.39±0.63.

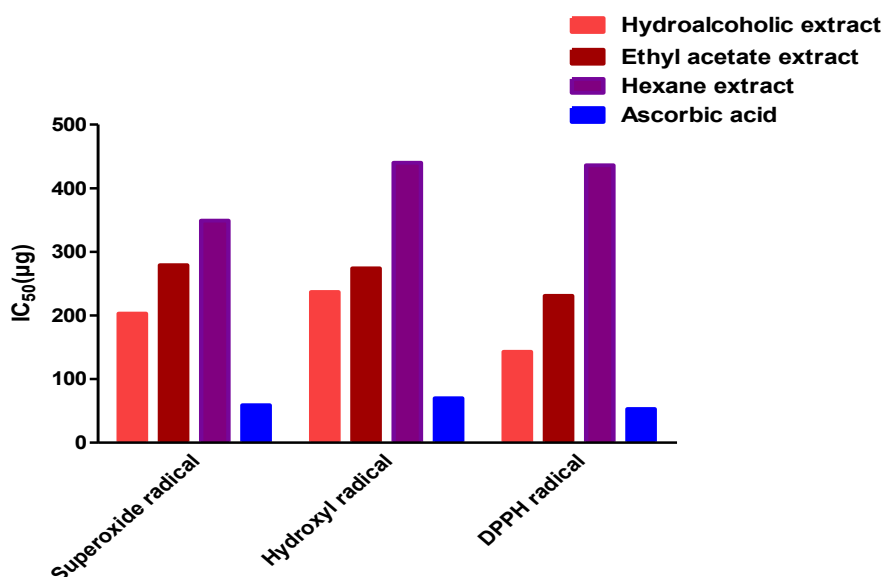
The mean IC<sub>50</sub> values for hydroxyl radical of hydroalcoholic, ethyl acetate and hexane extracts of *A. marina* were found to be 237µg, 274µg and 440µg respectively. The mean IC<sub>50</sub> value of ascorbic acid was found to be 70µg. Among all extracts hydroalcoholic extract at a concentration of 640µg showed the better scavenging activity on hydroxyl free radical i.e. 63.56±0.36.

The mean IC<sub>50</sub> values for DPPH radical of hydroalcoholic, ethyl acetate and hexane extracts of *A. marina* were found to be 143µg, 231µg and 436µg respectively. The mean IC<sub>50</sub> value of ascorbic acid was found to be 53.1µg. Among all extracts hydroalcoholic extract at a concentration of 640µg showed the better scavenging activity on DPPH free radical i.e. 73.23±0.56.

The order of activity in the following manner: ascorbic acid > hydroalcoholic extract > ethyl acetate extract > hexane extract.

**Table 6.** 50% Inhibition concentrations (IC<sub>50</sub>) of different extracts of *A. marina* against Superoxide, Hydroxyl and DPPH radicals.

Name of the extract	50% Inhibition Conc (IC <sub>50</sub> )		
	Superoxide radical	Hydroxyl radical	DPPH radical
Hydroalcoholic extract	203	237	143
Ethyl acetate extract	279	274	231
Hexane extract	349	440	436
Ascorbic acid	59	70	53.1



**Fig. 5.** 50% Inhibition concentrations ( $IC_{50}$ ) of different extracts of *A. marina* against Superoxide, Hydroxyl and DPPH radicals.

#### 4. DISCUSSION

The rural and tribal women are well acquainted with the wild resources around them. They seem to depend on plants for curing various diseases including abortion, sterility, conception disorders, menstrual troubles, leucorrhoea etc prevailing among them. However many of the uses of plants as abortifacient and antifertility herbals documented from the area, have also been reported from different countries by various researchers and also people use the plants for the diseases they suffer (abortion, sterility, conception disorders, menstrual troubles, leucorrhoea), it is very difficult to judge the effectiveness of plants [13, 22, 25, 26]. This stimulated to the present study i.e the isolation of bioactive compounds and evaluation of antioxidant and abortifacient activity of *A. marina* leaves extracts with using different solvents which has been used by the tribal people for abortion [27-29].

The effect of different extract of *A. marina* on the duration of different phases of estrus cycle were studied in rats. During estrus cycle many physiological, biochemical, morphological, and histological changes occur in the ovaries. Follicular growth and ovulation are regulated by endocrine (follicle stimulating hormone, luteinizing hormone, and prolactin) and ovarian hormones (such as progesterin, estrogen, and androgen). Ovarian hormones are produced by different cell types of the ovary like granulosa cells of the mature follicles and the corpus luteum [30]. Imbalance in these hormones leads to irregularity in the ovarian functions and duration of the estrus cycle [31].

In our study, the number of days the rats of groups II and III spent in the estrus cycle showed a significant decrease in the median duration of estrus and metestrus phases (as compared to the control group). Reduction in the estrus and metestrus phases indicates nonavailability of the matured graafian follicles or nonmaturation of secondary follicles. Therefore, ovulation was inhibited. There was an increase in the mean number of days the rats spent in proestrus phase in groups II and III, which was significant as compared with the

control group. The prolongation of the proestrus phase indicates that maturation of the follicles in the preovulatory phase was delayed leading to nonmaturation of graafian follicles. This was due to nonavailability of estrogen produced by the granulosa cells which is essential for the maturation and differentiation of the ovarian follicles, or imbalance in the endogenous steroid, protein, and hormones.

In the present study abortifacient and anti oxidant activities were screened for different extracts of *A. marina*. The different extracts of *A. marina* showed concentration dependent percentage inhibition on tested free radicals (Superoxide, Hydroxyl and DPPH). The free radicals are produced in different metabolic process of the body and they can damage a wide range of biomolecules such as proteins, DNA and amino acids in the body [30, 32-35]. Recently, many researchers have been reported that many medicinal plants possess more potential antioxidant activity and their phytochemical constituents (Phenolic acids, flavonoids and tannins, etc) have potential biological activities. The present studies have shown that the extracts of *A. marina* have free radicals scavenging ability. Among all the extract of plants the hydroalcoholic extract showed the better percentage inhibition on free radicals.

The abortifacient activity of *A. marina* plant extracts (at 250 and 500 mg/kg b.w) was screened using rats by measuring the preimplantation loss, postimplantation loss and percentage of survival ratio of live fetuses. The extracts were found more active in preimplantation loss, postimplantation loss at dose of 500mg/kg b.w when compared with control group. Abortifacient plants, block, alter, or interfere in the production of hormones (estrogen and progesterone). The lining of the uterus does not grow enough to be supportive or nourishing to a fertilized egg, thus preventing implantation [22, 25, 26, 31]. The abortifacient activity (based on pre and post implantation loss) of tested different extracts of *A. marina* was may be due to the above said reasons. From the results obtained in the present study, it could be concluded that the different extracts of selected plant extracts possesses abortifacient activity and justifies the folkloric claim of as an abortifacient. Different bioactive compounds have been conformed abortifacient activity in animal models [7]. Therefore, it is assuming that based on phytochemical analysis of selected plant different phytochemicals (Alkaloids, Phenolics, Steroids and Glycosides etc.,) present in this plant may act either alone or on combination responsible for the observed abortifacient activity in the present study. Furthermore, the mechanism of abortifacient activity of selected plant extracts is wrathful study to identify the bioactive principle of abortifacient activity.

## 5. CONCLUSION

The current study was intended to evaluate abortifacient and antioxidant activity of *Avicennia marina* leaves extracts. Abortifacient activity was evaluated in rats, compared with standard drug (Mifepristone) and antioxidant activity was evaluated by using three free radicals (Superoxide, Hydroxyl and DPPH) compared with Ascorbic acid. The extracts were showed pre implantation loss, post implantation loss of implantations and decreased the survival ration of fetuses. Among all extracts hydroalcoholic extract showed better activity. The selected plant extracts showed concentration dependent percentage inhibition of free radicals.

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