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Abortifacient and antioxidant activities of different extracts of *Musa rosacea*

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ABSTRACT

Objective: To evaluate abortifacient and antioxidant activity of *Musa rosacea (M. rosacea)*. **Methods:** 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). **Results:** The extracts showed preimplantation loss, postimplantation 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 four extracts hydroalcoholic extract showed better activity with IC_{50} values on superoxide, hydroxyl and DPPH radicals were 180 μg, 218 μg and 116 μg. **Conclusion:** From the results obtained during the study it could be concluded that *M. rosacea* extracts have abortifacient and antioxidant components and the results support its folklore usage as abortifacient plant. Further is necessary for isolation and characterization of bioactive molecules which are responsible for these activities.

1. Introduction

Herbs have their own methods of affecting the body, each is different, unique. Herbs will be most effective when the user knows how to use them and when to use what.

The use of herbs (plants) is very common in different tribal communities of the world in accordance with the situation and necessity. The use of surgical method and drugs like synthetic steroidal contraceptives (e.g. mifepristone, misoprostol), prostaglandins and antiprogestins (in medical method), but they are often marked with serious side effects such as gastrointestinal problems, severe and painful uterine contractions, systemic illness, permanent sterility or even death. Therefore, the screening of plants with abortifacient activity and the subsequent identification and characterization of the active principle(s) will be a useful guide towards the formulation of cheaper, affordable contraceptive with reduced toxicity. In recent days many researchers reported the folklore claims of abortifacient plants used by the people scientifically [1-4].

On ethnobotanical survey on herbals which are used by the tribal people in araku valley region of Visakhapatnam District, Andhra Pradesh, India claimed to be used to abortion was *Musa rosacea (M. rosacea)* [5]. 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 *M. rosacea* 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 katika water falls, 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 Ethanol (70%)) using maceration process. Then the extracts were used for screening abortifacient activity.

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±2)℃ with an alternating 12 h light-dark cycle and relative humidity of (50±15) %, one week before the start and also during the experiment as per the rules and regulations of the Institutional

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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 *M. rosacea* (at 250 and 500 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 tetrozolium was purchased from Sisco Research Laboratories Pvt Ltd., Mumbai. Riboflavin was purchased from Loba Chemie Pvt Ltd., Bombay.

Acute toxicity study was conducted according OECD Guide lines No.423. After fasting overnight, mice were administered with extracts of *M. rosacea* in a single dose up to the highest dose of 2 000 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.

2.4. Abortifacient activity

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 2 mL of 1% gum acacia (*p.o.* daily). Groups II received standard drug (Mifepristone) and groups III to VIII received different extracts of *M. rosacea* (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 18th 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 [1, 6-8].

The following parameters will be observed: Number of corpora lutea, number of implantations, implantation index, number of live foetuses, number of dead foetuses,and survival ratio. Inplantation index and survival ratio were calculated using the following formulae:

$$\text{Implantation index} = \frac{\text{Total number of implantation sites}}{\text{Number of corpora lutea}} \times 100$$

$$\text{Survival ratio} = \frac{\text{Number of live foetuses}}{\text{Number of live foetuses+ Number of dead foetuses}} \times 100$$

2.5. In vitro antioxidant activity [9]

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

2.5.1. 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 [10].

2.5.2. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity is commonly used to evaluate the free radical scavenging effectiveness of various antioxidant substances [11]. Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated from the Fe^{2+}/EDTA/H_2O_2 system (Fenton reaction). The hydroxyl radical attacks deoxyribose, which eventually results in the formation of thiobarbituric acid reacting substances (TBARS).

2.5.3. DPPH radical scavenging activity

The scavenging activity for DPPH free radicals was measured according to the procedure described by Braca *et al.*, [12]. 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 [13].

3. Results

3.1. Abortifacient activity of different extracts of M. rosacea

Administration of 250 and 500 mg/kg b.w. of the different extracts of *M. rosacea* decreased the number of live fetus between 16.67% to 44.15% (survival ratio), while no live fetus was observed in the standard drug treated animals. The hydroalcoholic extract at a dose of 500 mg/kg b.w. show significant reduction in survival of foetuses i.e. 16.67%, the preimplantation and postimplantation loss were 50.81% and 83.33%.

The implantation index of all extracts was not more different from each other in all the treated groups, but the percentage of survival ratio and postimplantation loss was different. The postimplantation loss was significantly shown by the hydroalcoholic extracts at both doses compared to other two extracts. There was significant difference in the preimplantation loss and the postimplantation loss among all extracts treated groups. The postimplantation loss was increased (nearly doubled) than preimplantation loss in the 250 and 500 mg/kg b.w of extract-treated animals, respectively. The results were showed in Table 1.

3.2. Antioxidant activity of M. rosacea

In the present study, hydroalcoholic, ethyl acetate and hexane extracts of *M. rosacea* were found to possess concentration dependent free radical scavenging activity on superoxide, hydroxyl and DPPH free radicals.

The mean IC_{50} values for superoxide radical of hydroalcoholic, ethyl acetate and hexane extracts of *M. rosacea* extracts were found to be 180 μg, 336 μg and 483 μg respectively. The mean IC_{50} value of ascorbic acid was found to be 59 μg. Hydroalcoholic extract at a concentration of 640 μg showed the better scavenging activity on superoxide free radical i.e. (72.8±0.2) (Table 2 and Figure 1).

The mean IC_{50} values for hydroxyl radical of hydroalcoholic, ethyl acetate and hexane extracts of *M. rosacea* were found to be 218 μg, 304 μg and 635 μg respectively. The mean IC_{50} value of ascorbic acid was found to be 70 μg. Among all extracts hydroalcoholic extract at a concentration of 640 μg showed the better scavenging

Table 1
Abortifacient activity of different extracts of *M. rosacea*.

S. no	Parameter	Control	Standard (Mifepristone)	Hydroalcoholic extrat		Ethylacetate extract		Hexane extract	
				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
1.	Number of corpora lutea	8.66±0.36	9.17±0.29	9.83±0.39	10.16±0.25	10.00±0.39	9.17±0.46	10.17±0.48	9.33±0.29
2.	Number of implantations	7.33±0.23	0.83±0.16	6.33±0.20	5.00±0.15	5.67±0.25	5.67±0.34	7.67±0.29	6.50±0.36
3.	Implantation index (%)	84.62	9.09	64.41	49.18	56.67	61.82	75.41	69.64
4.	Number of live foetuses	7.33±0.23	0.00	1.67±0.09	0.83±0.07	1.67±0.14	1.17±0.12	2.33±0.14	1.50±0.17
5.	Number of dead foetuses	0.00	0.83±0.16	4.67±0.14	4.17±0.13	4.00±0.18	4.50±0.29	5.33±0.17	5.00±0.26
6.	Preimplantation loss (%)	15.38	90.91	35.59	50.81	43.33	38.18	24.59	30.36
7.	Postimplantation loss (%)	0.0	100	73.68	83.33	70.59	79.41	69.57	76.92
8.	Survival ratio (%)	100.00	0.00	26.32	16.67	42.71	29.95	44.15	40.37

activity on hydroxyl free radical i.e. (66.84±0.40) (Table 2 and Figure 2).

The mean IC_{50} values for DPPH radical of hydroalcoholic, ethyl acetate and hexane extracts of *M. rosacea* were found to be 116 μg, 245 μg and 516 μg respectively. The mean IC_{50} value of ascorbic acid was found to be 53.1 μg. Among all extracts hydroalcoholic extract at a concentration of 640 μg showed the better scavenging activity on DPPH free radical i.e. (74.40±0.80) (Table 2 and Figure 3).

Table 2
50% Inhibition concentrations (IC_{50}) of different extracts of *M. rosacea* against superoxide, hydroxyl and DPPH radicals.

Name of the extract	50% inhibition conc (IC_{50})		
	Superoxide radical	Hydroxyl radical	DPPH radical
Hydroalcoholic extract	180	218	116.0
Ethyl acetate extract	336	304	245.0
Hexane extract	483	635	516.0
Ascorbic acid	59	70	53.1

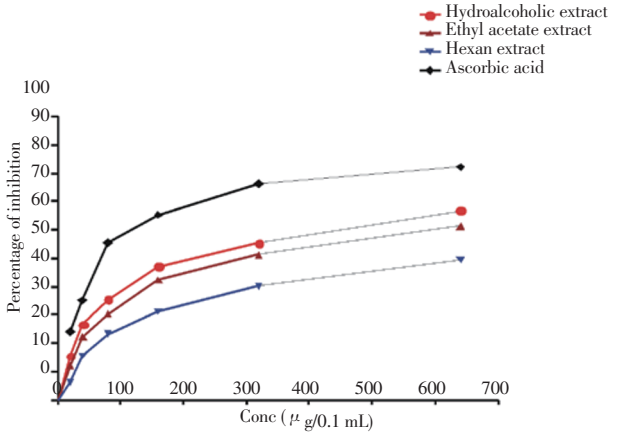

Figure 2. Concentration dependent percentage inhibition of different extracts of *M. rosacea* on hydroxyl radical.

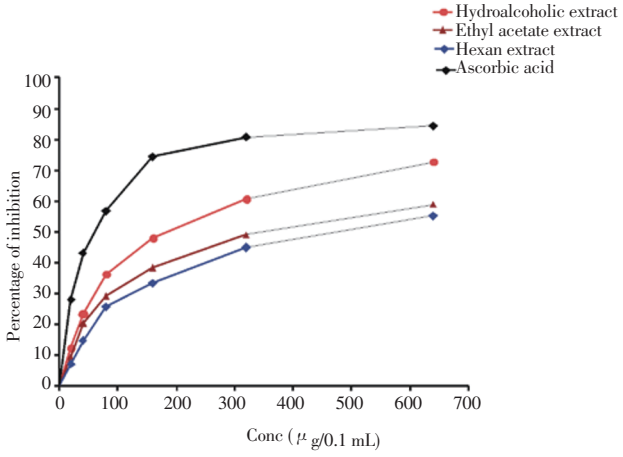

Figure 1. Concentration dependent percentage inhibition of different extracts of *M. rosacea* on superoxide radical.

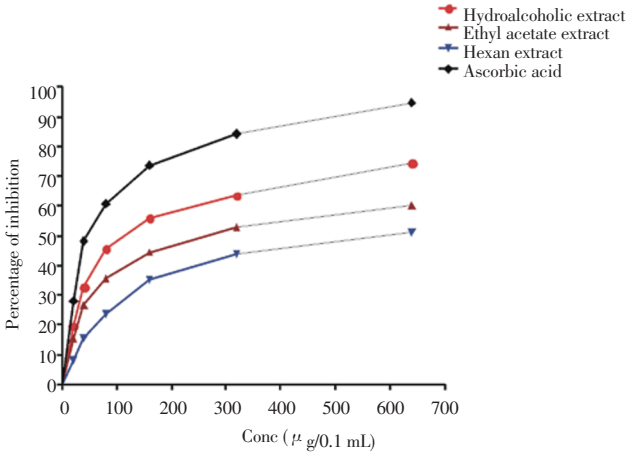

Figure 3. Concentration dependent percentage inhibition of different extracts of *M. rosacea* on DPPH radical.

The *M. rosacea* extracts showed better scavenging activity on DPPH free radical than Superoxide and hydroxyl free radicals. Among all *M. rosacea* extracts, the hydroalcoholic extract showed better activity.

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

4. Discussion

In the present study abortifacient and anti-oxidant activities were screened for different extracts of *M. rosacea*. The different extracts of *M. rosacea* 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 [14-18]. 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 *M. rosacea* 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 *M. rosacea* 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 foetuses. The extracts were found more active in preimplantation loss, postimplantation loss at dose of 500 mg/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 [19-22]. The abortifacient activity (based on pre and post implantation loss) of tested different extracts of *M. rosacea* 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 [1]. Therefore, it is assuming that based on phytochemical analysis of selected plant [23] 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 worthful study to identify the bioactive principle of abortifacient activity.

Conflict of interest statement

We declare that we have no conflict of interest.

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