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***Research Article***

# Phytochemical investigation and evaluation of anthelmintic and wound healing activity of various bark extracts of *Melia azedaracha* Linn. (Meliaceae)

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## ABSTRACT

***Background***: Medicinal plants are widely used by the traditional medicinal practitioners to cure various diseases due to their world-wide availability and less side effect. Today due to increasing side effects of many synthetic drugs, the demand of natural drugs for the treatment of human disease or ailments is preferred. Hence, research were going on natural drugs day to day to find out new molecule and more potent drugs which can be more helpful to human beings at present and in future for treatment of their malady. The present study is to examine the anthelmintic and wound healing potentials of various bark extracts of *Melia azedarach* Linn.

***Methods***: Phytochemical investigation was done by using various chemical analysis and standard methods. The anthelmintic activity was done by using Indian earthworms *Pheretima posthuma* as test worm, Albendazole (60 mg/mL) was used as a reference standard and the wound healing activity was done by using different wound model i.e. excision and incision wound model.

***Results***: Phytochemical study showed presence of alkaloids flavonoids, steroids, terpenoids, anthraquinones, tannins, saponins and acids. Among all the extracts, ethanol and petroleum ether showed dose dependent & significant anthelmintic activity, petroleum ether showed better activity as compared to reference drug albendazole. The ethanolic extract showed significant increase in wound contraction and formation of scar in excision wound model. The extract showed significant increase in the breaking strength of re-sutured incision wound as compared to control group ($p$<0.05). The result of the present study indicated that petroleum ether showed better anthelmintic activity as compared to reference drug albendazole. The ethanolic extract of *M. azedarach* has more significant wound healing property as compared to other extracts in excision and incision wound model.



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## 1. Introduction

*Melia azedara* is a species of deciduous tree in the mahogany family, Meliaceae, which is an evergreen tree, cultivated in various parts of the Indian subcontinents. It is native in Pakistan, India, Indochina, Southeast Asia and Australia. It is widespread and naturalized in most of the tropics and subtropical countries (Burks, 1992). *M. azedarach* L. is a small to medium sized deciduous tree. It grows to a height of 5 to 15m tall and 30 to 60cm in diameter. The plant is characterized by the presence of a spreading, dense and dark green crown. Its bark is dark brown in color, relatively smooth, and fissured. The leaves are alternate, leaflets are short stalked and thin, hairless, dark green and relatively pale. Flowers are white with purple stripes and are characterized by the presence of a typical fragrance. Fruits or berries are yellow, round, smooth, and fleshy. Dried fruits are hard with 4 to 5 seeds (Burks, 1992). All part of the plants like Leaf, bark, flower and seeds were used for treatment of various ailments of human as well as animals. Leaf juice is used as anthelmintic, diuretic, expectorant, vermifuge and their decoction were used for treatment of various ailments. Decoction of bark is used in paroxysmal fever to relieve thirst, nausea, vomiting, general debility, and loss of appetite and skin diseases (Dhiman, 2003; Sharma et al., 2003). Flowers have astringent, diuretic, resolvent, deobstruent properties. Seeds are considered anthelmintic, expectorant, aphrodisiac and are useful in typhoid fever, also prescribed in rheumatism. Seed oil is used in skin diseases. Roots are astringent and emmenagogue. These are useful in sciatica, lumbago, piles, cough, asthma, ulcers, wounds, diabetes, intermittent fever, post labor pain in uterus, amenorrhea and in leuoderma (Warrier et al., 1995). Fruits are considered anthelmintic, diuretic, emollient and purgative (Rani et al., 1999). *M. azedarach* contains a number of organic molecules like flavo-

-noids, terpenoids, steroids, acids and anthraquinones (Sen and Batra, 2012).

## 2. Materials and methods

### *2.1. Collection and authentication of plant*

The bark of *M. azedarach* were collected from the campus of Jeypore college of pharmacy, Jeypore, Koraput district.(India) in the month of September 2016. The plant was identified, confirmed and authenticated by Mr. Biju Patnaik Medicinal Plants Garden and Research Centre, Jeypore, Koraput (District), Orissa (Letter No. MJ/SS/P-427/16, dated 12.9.2016).

### *2.2. Preparation of extracts*

After authentication, the collected plant parts and barks were separated from undesirable materials and washed thoroughly with sterile water several times. It was shade dried for one week and then dried in an oven to make it suitable for grinding. The coarse powder was successively extracted in Soxhlet apparatus with solvents such as petroleum ether, ethyl acetate, *n*-butanol and ethanol. A total amount of 650 g coarse powder was extracted with 1000 mL of solvent. 10 cycles were run to obtain thick slurry. Each slurry was then concentrated under reduced pressure to obtain the crude extract. The crude extracts were kept in closed air tight containers under cool and dark place for further study (Kokate et al., 2007; Trease and Evans, 1989).

### *2.3. Drugs and chemicals*

Albendazole was procured as gift sample from Sri Pharmacare, Mumbai, India. The ethanol AR and ethyl acetate AR 60-80°C (Emsure® ACS) were procured from Merck Pvt. Ltd., Navi Mumbai, Maharashtra, India. *n*-butanol GR 80°C, petroleum ether AR 40-60°C, Loba Chemie Pvt. Ltd., Mumbai, India. All other chemicals reagents used in present work were procured from authorized dealer.

### *2.4. Animals*

Wistar strain of albino rats weighing between 180-250g was used. They were housed in standard conditions of temperature (25±2°C), 12 hours light per day cycle, relative humidity of 45-55% in animal house of Jeypore College of Pharmacy. They were fed with standard pellets of food and water. Animals were kept and all operation on animals was done in aseptic condition. All the studies conducted were approved by the Institutional Animal Ethical Committee (1906/PO/Re/S/16/CPCSEA), Jeypore College of pharmacy, Jeypore, Odisha according to prescribed guide-lines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

### *2.5. Preliminary phytochemical investigation*

The crude ethanol extract of the bark of *M. azedarach* were subjected to preliminary phytochemical analysis in order to detect the presence of various groups of phytoconstituents by carrying out the standard procedure for chemical analysis (Harbone, 1973; Morton and Malon, 1972).

### *2.6. Anthemintic activity*

#### *2.6.1. Worm collection and authentication*

The anthelmintic activity was evaluated on adult Indian earthworm *Pheretima posthuma* due to its resemblance anatomically and physiologically with the intestinal round worm parasite of human being. Indian earthworms were obtained from vermiculture area and were identified by the V.D, College (Autonomous), Department of Zoology, Jeypore, Koraput, Odisha, India.

#### *2.6.2. Preparation of Test sample*

The test samples were prepared by dissolving and suspending 2.5 g of each extract in 25 mL of distilled water to obtain a stock solution of 100 mg/mL. From this stock solution, different dilutions were prepared to get concentration range of 50 and 100 mg/mL.

#### *2.6.3. Anthelmintic Assay*

The anthelmintic activity of bark extract of *M. azedarach* was evaluated on adult Indian earthworms by the reported methods with minor modification. The assay was performed on adult Indian earthworm *Pheretima posthuma* as its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings and also easy availability earthworms have been used widely for the initial evaluation of anthelmintic activity. The *in vitro* anthelmintic activity was determined by releasing into 10 mL of desired formulation containing three different concentration, each of crude extract i.e. petroleum ether extract, ethyl acetate, *n*-butanol and ethanol extract (50 and 100 mg/mL in distilled water) were prepared. Albendazole (60 mg/mL) was used as reference standard while distilled water used as control and six worms (same type) were placed in it. Observations were made for the time taken to paralysis and/or death of individual worms. Paralysis was said to occur when the worms do not revive even in normal saline water. Death was concluded when the worms lose their motility followed with fading away of their body color or not moved when dipped in warm water at 50ºC (Mali et al., 2007; Ghosh et al., 2009; Vigar, 1984).

### *2.7. Wound healing activity*

#### *2.7.1. Excision wound*

For the excision wound study, animals were divided into 5 groups of six rats in each group. Group-I served as control and applied with Vaseline; Group-II, Group-III, Group-IV and Group-V were treated with ethanol, ethyl acetate, *n*-butanol and petroleum ether extracts respectively. An impression was made on the dorsal thoracic central region 5 mm away from the ears, by using a round seal of 2.5 cm diameter as described by Morton and Malone (Ehrlich and Hun, 1969). The skin of the impressed area was excised to the full thickness to obtained area of about 500 $mm^2$ under light ether anaesthesia in aseptic condition. The animals were housed individually. The methanol extracts in simple ointment base (5% w/w) were applied on the wound once a day for 18 days starting from the day of wounding. The percentage wound closure was observed on $4^{th}$, $8^{th}$, $12^{th}$, $16^{th}$ and $18^{th}$ post wounding day. Epithelization time

(in days) and the size of scar area was noted.

*2.7.2. Incision wound*

Incision wound model was performed according to Ehrlich and Hunt (Lee, 1968). The animals were divided into 5 groups of six rats in each group, and kept in separate cage. Group-I served as control, received only 2% gum acacia suspension (1 mL/kg, p.o). Ethanol, ethyl acetate, *n*-butanol and petroleum ether extracts (250 mg/kg) were given orally once a day to group-II, III, IV and V respectively for 10 days. Under light ether anesthesia, the animals were secured to operation table in its natural position. Two paravertebral straight incisions of 6 cm each were made through the entire thickness of the skin, on either side of the vertebral column with help of sharp blade. Removal of the sutures was done on 8th post wounding day. Tensile strength was determined on both wounds by continuous constant water flow technique of Lee (1968).

*2.8. Statistical analysis*

Results are reported as Mean ± SEM. Statistical analysis was done using ANOVA (Tukey-Multiple Comparison Test). When probability '*p*' was less than 0.05 was considered as significant (Bolton, 2004).

## 3. Results and Discussion

*3.1. Phytochemical Investigation*

The preliminary phytochemical screening of the different solvent extracts of *M. azedarach* bark showed the presence of alkaloids, flavonoids, steroids, terpenoids, Anthraquinones, tannin, saponins and acids. The ethanol and *n*-butanol extract yielded all the phytochemicals, but anthraquinones and acid were absent in both petroleum ether and ethyl extract (Table 1).

*3.2. Anthelmintic activity*

The ethanol and petroleum ether extract of bark of *M. azedarach* showed significant anthelmintic activity at higher concentration (100 mg/mL). The extract showed a dose dependent activity like shortest time of paralysis and death with (100 mg/mL) concentration. The ethanol extract of *M. azedarach* bark caused paralysis in 23.18 min and death at 43.22 min while petroleum ether extract showed paralysis in 24.12 min and death at 53.14 min. As compared to the reference drug albendazole showed the same at 27.13 min and 57.23 min respectively which were shown in (Table 2).

**Table 1.** Phytochemical screening of the different bark extracts of *M. azedarach*

| Extracts | Phytochemicals | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Alkaloids | Flavonoids | Steroids | Terpinoids | Anthraquinones | Tannins | Saponins | Acids |
| Ethanol | ++ | +++ | +++ | +++ | ++ | ++ | ++ | + |
| Ethyl acetate | + | + | + | ++ | - | + | + | - |
| *n*-butanol | + | + | ++ | ++ | + | + | ++ | + |
| Petroleum ether | + | ++ | + | + | - | ++ | + | - |

**Indications:** +++ High; ++ moderate; + poor; - absent

**Table 2**. Anthelmintic activity of *M. azedarach* bark extracts

| *Treatment vehicle* | *Concentration* | *Time taken (in min)* | |
|---|---|---|---|
| | | *For paralysis* | *For death* |
| Ethyl acetate extract | 50 mg/mL | 37.11 ± 26 | 83.28 ± 0.12 |
| | 100 mg/mL | 33.16 ± 0.30 | 73.16 ± 0.17 |
| Ethanol extract | 50 mg/mL | 26.14 ± 0.40 | 55.28 ± 0.14 |
| | 100 mg/mL | 23.18 ± 0.16 | 43.22 ± 0.80 |
| *n*-butanol extract | 50 mg/mL | 31.14 ±0.04 | 77.23 ± 17 |
| | 100 mg/mL | 28.11 ± 0.22 | 68.16 ± 0.06 |
| Petroleum ether | 50 mg/mL | 24.12 ± 0.18 | 53.14 ± 0.23 |
| extract | 100 mg/mL | 21.07 ±0.33 | 42.33 ± 0.06 |
| Albendazole | 60 mg/mL | 27.13 ± 0.24 | 57.23 ± 0.11 |
| Control Vehicle | - | - | - |

*Values are mean ± SEM (n=6)*

**Table 3.** Effect of bark extracts of *M. azedarach* on the breaking strength in incision wound

| Sl no. | Group | Breaking strength |
|---|---|---|
| 1 | Control | 273.12 ± 21.09 |
| 2 | petroleum ether | 327.26 ± 14.23 |
| 3 | ethyl acetate | 347.33 ± 13.43 |
| 4 | *n*-butanol | 351.12 ±141.21 |
| 5 | ethanol | 438.76± 18.27* |

Values are mean ± S.E.M (n=6)* p<0.05 vs control

## 3. Results and Discussion

*3.1. Phytochemical Investigation*

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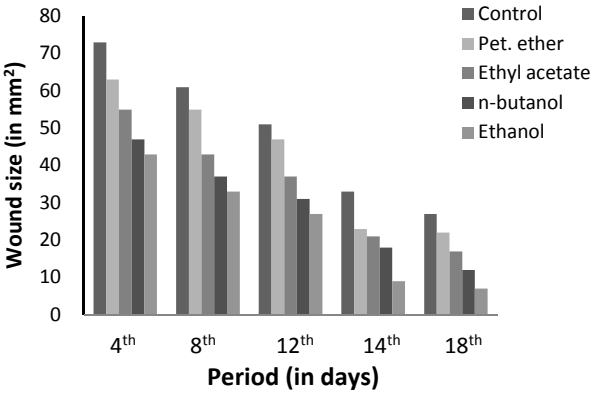


**Figure 1.** Effect of *M. azedarach* bark extracts on excision wound model

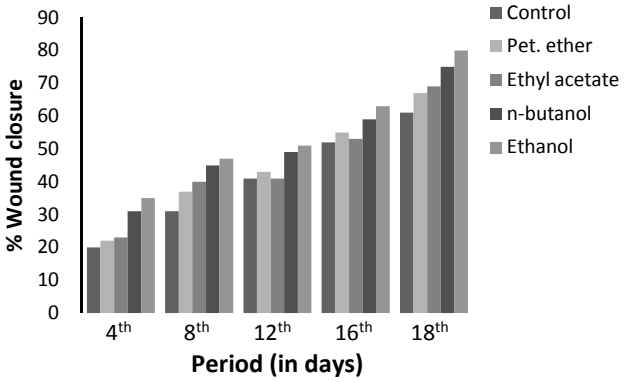


**Figure 2.** Effect of *M. azedarach* bark extracts on wound contraction in excision wound model

*3.3. Wounding healing activity*

In the study using excision wound model, animals treated with ethanol extract *M. azedarach* bark showed significant decrease in epithelization period as evidenced by shorter period for fall of eschar as compared to control group ($p$<0.05) (Figure 1). The extract also facilitated the increase in rate of wound contraction than control group. Petroleum ether extract treated animal (Group-II) showed wound contraction by 65.17%. Ethyl acetate extract treated animals (Group-III) showed wound contraction by 71.23%. *n*-butanol extract treated animal (Group-IV) showed wound contraction by 73.27%, where as ethanol extract treated animal (Group-V) showed wound contraction by 83.33% as compared with the control (Group-I) by 61.22% in all the extract (Figure 2). The results of present study reveals that ethanol bark extract of *M. azedarach* possess a prominent healing activity in incision wound model. This was demonstrated by significant increase in the skin tensile strength in methanol extract treated groups ($p$<0.05) on 18th post wounding day (Table 3).

## 4. Conclusion

From the phytochemical analysis, the presence of tannins as one of the chemical constituent said to possess anthelmintic activity. Among all the extracts petroleum ether and ethanol extract showed dose dependent and significant anthelmintic activity, with petroleum ether extract showing better activity as compared to reference drug albendazole. As the bark extract showed activity against the earthworms used in the study. Further studies were underway to attempt the isolation of the active compound present in the crude extract of *M. azedarach* and to establish the mechanism of action for anthelmintic activity. Also, the present study suggested that local application and systemic administration of ethanol extract of the bark has shown more significant wound healing activity in excision and incision wound models and supports the popular use of plant to open wound in folk medicine. The wound healing property of *M. azedarach* has been attributed to its antimicrobial effects. The presence of phytoconstituents like flavonoids, terpenoids, saponins, phenols, steroids and tannins either individually or combined together may exhibit the synergistic effect towards healing of wounds.

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## Conflict of Interest

Authors report no conflict of interest.

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