

Evaluation of wound healing effect of petroleum ether and methanolic extract of *Abelmoschus manihot* (L.) Medik., Malvaceae, and *Wrightia tinctoria* R. Br., Apocynaceae, in rats

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RESUMO: “Avaliação do efeito de cicatrização dos extratos de éter de petróleo e metanol de *Abelmoschus manihot* (L.) Medik., Malvaceae, e *Wrightia tinctoria* R. Br., Apocynaceae, em ratos”. Nos últimos anos, o estresse oxidativo e radicais livres têm sido implicados na cicatrização. *Abelmoschus manihot* (L.) Medik., Malvaceae e *Wrightia tinctoria* R. Br., Apocynaceae, plantas utilizadas na medicina Ayurveda, possuem propriedades antiinflamatórias e antimicrobianas. O presente estudo foi realizado para avaliar o potencial dos extratos de éter de petróleo e metanólico na cicatrização de feridas em ratos Wistar. Os ratos foram divididos em seis grupos com seis animais cada. O grupo 1 foi utilizado como controle, o grupo 2 recebeu a droga padrão e os outros quatro grupos foram tratados com duas doses diferentes de cada um dos extratos de *A. manihot* e *W. tinctoria*. Os parâmetros de cicatrização foram avaliados através da incisão feridas em ratos tratados com extrato, padrões e controles. Ambas as doses dos extratos de éter de petróleo e metanólico aumentaram significativamente força de ruptura da ferida quando comparados ao grupo controle.

Unitermos: *Abelmoschus manihot*, *Wrightia tinctoria*, atividade cicatrizante.

ABSTRACT: In recent years, oxidative stress and free radicals have been implicated in impaired wound healing. *Abelmoschus manihot* (L.) Medik., Malvaceae, and *Wrightia tinctoria* R. Br., Apocynaceae, plants widely used in Ayurveda, possesses anti-inflammatory and antimicrobial properties. The present study was undertaken to assess the potential of petroleum ether and methanol extracts in wound healing in Wistar albino rats. The rats were divided into six groups of six animals each. Group 1 is normal wounded control, group 2 received standard drug and the other four groups were treated with two different doses each of petroleum ether and methanol extract of *A. manihot* and *W. tinctoria*. The wound healing parameters were evaluated by using incision wounds in extract-treated rats, standard and controls. Both the doses of petroleum ether and methanol extract significantly increased wound breaking strength when compared with the control group.

Keywords: *Abelmoschus manihot*, *Wrightia tinctoria*, wound healing activity.

INTRODUCTION

Acute wounds normally heal in a very orderly and efficient manner characterized by four distinct but overlapping phases: Hemostasis, inflammation, proliferation, and remodeling (Robert & Melissa, 2004). Approximately 225,000 spinal chord injury patients in US, 60% out of these patients develops pressure ulcers and annual cost estimate ranges from \$14,000 to \$25,000 per patient. Therefore national expenditure for costs related to the care of patients with pressure ulcer is over \$1.3 billion per year (Cockbill, 2002). Wound, one of the common clinical conditions has been targeted for exhaustive investigation so as to identify the ways and means of

promoting the healing process. Wound healing involves a complex series of interaction between different cell types, cytokines mediators and the extracellular matrix (Ehrlich & Hunt, 1968).

Abelmoschus manihot (L.) Medik., Malvaceae (Anonymous, 1976) is a large annual erect hairy plant, 1.2-1.8 m. high. It is native to China, introduced into India, near Calcutta and in coastal areas of Maharashtra. The plants mucilage contains polysaccharides and proteins (Kirtikar & Basu, 1994). The flower contain quercetin-3-robinoside, quercetin-3'-glucoside, hyperin, myrecetin and anthocyanins (Lai et al., 2009). The saturated acids and liquid acids such as linoleic and oleic acids were isolated from the seed fat and unsaponifiable matters. The different

chromatographic methods have been developed on the flavones present in the plant (Liang et al., 2007, Li et al., 2006). The ethanol extract of flower was screened for antiviral activity, and it was observed that the hyperoside shown significant anti HBV activity (Linlin et al., 2007). The flavones present in the plant showed preventive effect in the injury (Liu et al., 2009, Wen & Chen, 2007). The leaves were tested on bone loss in ovariectomised rats and it was observed that it was able to prevent the ovariectomy - induced femoral osteopenia (Puel et al., 2005) modulatory effect of total flavone of *Abelmoschus manihot* (TFA) on NMDA-activated current (*I*_{NMDA}) was investigated in cultured rat hippocampal neurons using the whole-cell patch-clamp technique. TFA rapidly and reversibly inhibited the *I*_{NMDA} in a concentration-dependent manner (Xin-ping et al., 2006).

Wrightia tinctoria R. Br., Apocynaceae, is a small deciduous tree, generally up to 1.8 m tall and often under 60 cm girth, sometimes up to 7.5 m high, distributed all over India. Four uncommon sterols, desmosterol, clerosterol, 24-methylene-25-methylcholesterol and 24-dehydropollinastanol, in addition to several usual phytosterols, were also isolated and identified (Akihisa et al., 1988). The wrightial, a new terpene and other phytoconstituents such as cycloartenone, cycloecalenol were isolated identified by fractionation of methanol extract of the immature seed pods (Ramchandra et al., 1993). The hexane extract of seed pods of *W tinctoria* was saponified and non saponifiable matter was fractionated with methanol gave a colourless substance, oleanolic acid (Rao & Rao, 1968). The five flavonoid compounds, indigotin, indirubin, tryptanthrin, isatin and rutin were isolated and identified from the leaves (Muruganadam et al., 2000). The unsaponifiable matter extracted from bark by petroleum ether extract was fractionated with methanol to yield a triterpenes like β -sitosterol and β -amyrin, wrightiadiene (Warrier, 1996). The ursolic acid has been also isolated from the chloroform extract of seed pods (Rao & Rao, 1966).

The bark is used as stomachic and in the treatment of abdominal pain and skin diseases (Shah et al., 1988), as antidysenteric, antidiarrhoeal and antihemorrhagic. The bark is used in flatulence and bilious affections. A decoction of the leaves and bark is taken as a stomachic and in the treatment of abdominal pain (Joshi et al., 1980). The dried and ground bark is rubbed over the body in dropsy. The plant is used in Ayurveda, Unani and Siddha medicines as astringent, febrifuge and tonic (Tomar & Singh, 1990). The seeds are said to possess antipyretic, analgesic, and anti-inflammatory activity (Ghosh et al., 1985).

Keeping in view the tremendous pharmacological activities and a wealth of available literature, *Abelmoschus manihot* and *Wrightia tinctoria*, may be utilized to alleviate the symptoms of a variety of diseases as evident from pre-clinical data (Nigam et al., 2006a). Thus it appears that different mechanisms like free radical scavenging,

metal chelation as well as immune modulation may act at different levels individually or in combination to bring about the wound healing effects of this medicinal plant.

From another viewpoint using animals for healing it is now universally acknowledged that maggot therapy can be used successfully to treat chronic, long-standing, infected wounds, which have previously failed to respond to conventional treatment. Such wounds are typically characterized by the presence of necrotic tissue, underlying infection and poor healing (Nigam et al., 2006b). Maggot therapy employs the use of freshly emerged, sterile larvae of the common green-bottle fly, *Phaenicia (Lucilia) sericata* and is a form of artificially induced myiasis in a controlled clinical situation. Maggot therapy has the following three core beneficial effects on a wound: debridement, disinfection and enhanced healing (Gupta et al., 2002).

MATERIAL AND METHODS

Plant material

The woody stem of *Abelmoschus manihot* (L.) Medik., Malvaceae, and *Wrightia tinctoria* R. Br., Apocynaceae, were collected during the month of May-June from the Toranmal Hills of Maharashtra. The plant was taxonomically identified by Professor Dr. D. A. Patil, HOD Botany Dept, SSVPS College, Dhule, North Maharashtra University, Jalgaon, M. S., India (Figure 1)



Figure 1. Aspect of *Abelmoschus manihot* (L.) Medik., Malvaceae and *Wrightia tinctoria* R. Br., Apocynaceae

Preparation of extracts

The woody stems of *A. manihot* and *W. tinctoria* were dried in shade and powdered. The woody stem powders (500 g) were extracted by petroleum ether and methanol using soxhlet apparatus at 60-75 °C. Extract was concentrated by evaporation (Suffness et al., 1978). The yield was about 1-5%. The semisolid extract was dissolved in saline by using gum acacia as a vehicle during the study.

Phytochemical evaluation of *Abelmoschus manihot* and *Wrightia tinctoria* (Harborne, 1998)

Test for steroid and triterpenoids

Salkowski reaction: A small quantity of petroleum ether extract of both the plants was dissolved in chloroform and a few drops of concentrated sulfuric acid were added to the solution. For extract of both the plants, a reddish color was seen in the upper chloroform layer.

Liebermann-Burchard reaction: A small quantity of petroleum ether extract of both the plants was dissolved in chloroform and a few drops of concentrated sulfuric acid were added to it followed by the addition of 2-3 drops of acetic anhydride. Solution for extracts of both the plants turned violet blue and finally green.

Test for flavonoids

Shinoda test: To dried methanol extract of both the plants, added 5 ml 95% ethanol, few drops concentrated HCl and 0.5 g magnesium turnings. Pink color was observed.

Preparation of ointment

The ointment of the petroleum ether and methanolic woody stem extracts of *A. manihot* and *W. tinctoria*, were prepared in concentration of 5% (w/w) and 10% (w/w) using simple ointment base.

Rats

Albino Wistar rats of either sex having weight of 200-250 g were procured from R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur. The rats were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 25-27 °C and 35-60% humidity). Standard palletized feed and water were provided *ad libitum*. The experimental protocol was subjected to scrutiny of Institutional Animal Ethical Committee for experimental clearance (N^o. IAEC/RCPIPER/UA/2009). The animals were divided into six groups of six animals each and were daily administered the extract of *A. manihot* and *W. tinctoria*.

Abelmoschus manihot

- Group I: Control group treated with simple ointment
- Group II: Positive (standard) control treated with Povidone Iodine Ointment
- Group III: 5% Ointment of woody stem petroleum ether extract of *A. manihot* (PEA)
- Group IV: 10% Ointment of woody stem petroleum ether extract of *A. manihot* (PEA)
- Group V: 5% Ointment of methanol extract of *A. manihot* (MEA)
- Group VI: 10% Ointment of methanol extract of *A. manihot* (MEA)

Wrightia tinctoria

- Group I: Control group treated with simple ointment
- Group II: Positive (standard) control treated with Povidone Iodine Ointment
- Group III: 5% Ointment of woody stem petroleum ether extract of *W. tinctoria* (PEW)
- Group IV: 10% Ointment of woody stem petroleum ether extract of *W. tinctoria* (PEW)
- Group V: 5% Ointment of methanol extract of *W. tinctoria* (MEW)
- Group VI: 10% Ointment of methanol extract of *W. tinctoria* (MEW)

Experimental procedure

The wounding procedure was carried out using 1 mL of ketamine (10 mg/kg body weight) anesthetized rats in incisional wound model. The ointment preparations and standard Iodine-Povidone ointment (CIPLADINE, Cipla Ltd.) were applied topically to the wound area. On the 9th post wounding day, the sutures were removed and skin breaking strength was measured on 10th post wounding day by continuous water flow technique.

Wound model incision wound

Two paravertebral incisions (6 cm long) were made through the full thickness of the skin on either side of the vertebral column (Ehrlich & Hunt, 1969). Wounds were closed with interrupted sutures, 1 cm apart. The sutures were removed on the 7th day. Wound breaking strength (WBS) was measured on the 10th post-wounding day (Lee, 1968).

Determination of wound breaking strength (Lee, 1968)

Rats were secured to the operation table and a line was drawn on either side of the wound 3 mm away from the wound. Two allice forceps were firmly applied on to the line facing each other. One of the forceps was fixed, while the other was connected to a freely suspended lightweight polypropylene graduated container through a string run over to a pulley. Water was allowed to flow

from the reservoir slowly and steadily into the container. A gradual increase in weight was transmitted to the wound site pulling apart the wound edges. As and when the wound just opened up, the water flow was arrested and the volume of water collected in the container (approximately equal to its weight) was noted. Three readings were recorded for a given incision wound and the procedure was repeated on the wound on the contralateral side. The average reading of the group was taken as an individual value of breaking strength. Mean value gives the breaking strength for a given group.

Acute toxicity studies

Healthy rats ($n=6$) were orally fed with increasing doses (400 mg/kg to 6 g/kg body weight) of extracts for 14 days.

Statistical Analysis

The results were analyzed using one way analysis

of variance (ANOVA) with Dunnett's multiple comparison test. p -values <0.05 were considered statistically significant.

RESULTS

The phytochemical screening of petroleum ether and methanolic extracts of both the plants showed positive test for steroids, triterpenoids and flavonoids. The doses up to 2 g/kg body weight did not produce any toxicity and mortality. The woody stem extracts of *Abelmoschus manihot* and *Wrightia tinctoria* showed significant wound healing activity in incisional wound model (Figure 2 and 3). In incisional wound model, (Table 1 and 2) indicates skin breaking strength (Tensile strength), the 5% and 10% petroleum ether and methanol extract ointment treated group shows significant ($p<0.01$) increase in tensile strength but standard treated animals showed better skin breaking strength as compared to the control group. Iodine-Povidone ointment used as standard for the study.

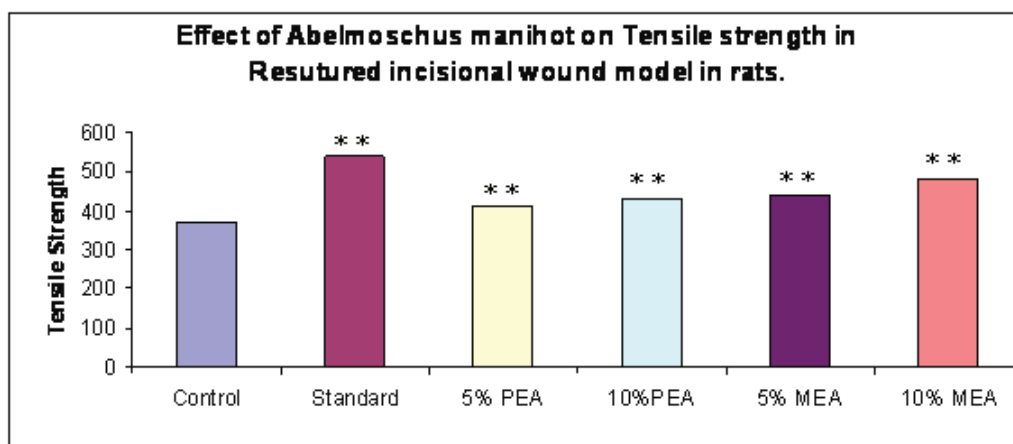


Figure 2. Wound healing activity of extracts of *Abelmoschus manihot* in incisional model.

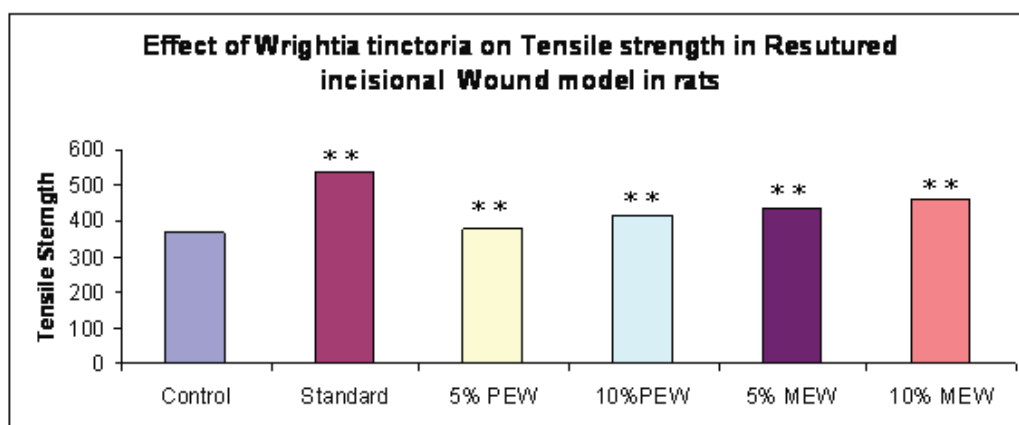


Figure 3. Wound healing activity of extracts of *Wrightia tinctoria* in incisional model.

Table 1. Effect of extracts of *Abelmoschus manihot* on tensile strength (g) in resutured incisional wound model in rats.

Animal N ^o	Control	Standard	5% PEA	10% PEA	5% MEA	10% MEA
1	367.5	514.5	391.0	453.0	431.5	469.5
2	370.5	549.5	408.5	435.0	448.5	487.5
3	379.5	537.0	410.0	429.5	457.5	490.0
4	364.0	557.5	389.5	432.5	443.0	473.5
5	380.0	526.0	416.5	419.0	442.5	471.5
6	340.0	540.5	415.5	415.0	435.5	479.0
Mean	366.9	537.5**	405.2**	430.7**	443**	478**
Std. Error	5.988	6.363	4.885	5.489	3.782	3.505

Values are Mean±S.E. (n=6); ANOVA followed by Dunnett's Multiple Comparison test. *p*-values are *p*<0.01** as compare with control. Where, 5% PEA- 5% ointment of petroleum ether extract of *Abelmoschus manihot*; 10% PEA- 10 % ointment of petroleum ether extract of *Abelmoschus manihot*; 5% MEA- 5% ointment of methanolic extract of *Abelmoschus manihot*; 10% MEA- 10 % ointment of methanolic extract of *Abelmoschus manihot*.

Table 2. Effect of extracts of *Wrightia tinctoria* on tensile strength (g) in resutured incisional wound model in rats.

Animal N ^o	Control	Standard	5% PEA	10% PEA	5% MEA	10% MEA
1	367.5	514.5	371.0	433.0	421.5	449.5
2	370.5	549.5	388.5	418.0	434.5	468.5
3	379.5	537.0	390.0	419.5	447.5	471.0
4	364.0	557.5	370.5	420.5	437.0	465.5
5	380.0	526.0	386.5	409.0	432.5	461.5
6	340.0	540.5	368.5	408.0	428.5	459.0
Mean	366.9	537.5**	379.2**	418.0**	433.6**	462.5**
Std. Error	5.988	6.363	4.139	3.717	3.555	3.160

Values are Mean±S.E. (n=6); ANOVA followed by Dunnett's Multiple Comparison test. *p*-values are *p*<0.01** as compare with control. Where, 5% PEW- 5% ointment of petroleum ether extract of *Wrightia tinctoria*; 10% PEW- 10 % ointment of petroleum ether extract of *Wrightia tinctoria*; 5% MEW- 5% ointment of methanolic extract of *Wrightia tinctoria*; 10% MEW- 10 % ointment of methanolic extract of *Wrightia tinctoria*.

DISCUSSION

The results from phytochemical screening showed that petroleum ether and methanolic extracts of woody stems of *Abelmoschus manihot* and *Wrightia tinctoria* mainly contain steroids, triterpenoids and flavonoids. There was reported that flavonoids, saponins like antioxidants showed significant wound healing activity. The enhanced wound healing may thus be due to the free-radical scavenging action of the plant, and the enhanced level of antioxidant enzymes in the tissues. In case of Resutured incisional wound model we were using petroleum ether and methanolic extracts of woody stems. In such type of model wound healing by primary intention. In this model, the increase in tensile strength of treated wounds may be due to the increase in collagen concentration and stabilization of the fibers (Udupa et al., 1995). Resutured wound was checked for the tensile strength. All the formulations i.e. 5% and 10% of both the plants showed significant increase in tensile strength. It may due to the enhanced action of myofibroblasts which are mainly responsible for the elasticity of tissue.

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