

Original Research Article

Wound healing activity of *Kaempferia rotunda* Linn leaf extract

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A B S T R A C T

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Kaempferia rotunda Linn is an Indian Ayurvedic plant. The present study reports that investigations were done on the aqueous and methanolic extract of *Kaempferia rotunda* Linn leaf by using the incision wounds and excision wounds modals study in rat. At the end of the day the study revealed that the aqueous extract and methanolic extract displayed significant wound healing activity and justifying the traditional claim.

Introduction

The wound may be defined as a loss or breaking of cellular and anatomic or functional continuity of living tissues. Healing of wound is a biological process that is initiated by trauma and often terminated by scar formation. The process of wound healing occurs in different phases such as coagulation, epithelization, granulation, collagenation and tissue remodeling. In India, there has been interest in the potential of medicinal plant for development of drugs with wound healing properties as taught in a popular form of Indian medicine known as Ayurveda (Jain *et al.*, 2006).

Two broad categories exist for the classification of wounds: chronic and acute. Acute wounds undergo a complex interactive process involving a variety of cell types that leads to a healed wound.

Conversely, chronic wounds have proceeded through portions of the repair process without establishing a functional anatomic result (Cohen *et al.*, 1999; Joan *et al.*, 2003).

Many medicinal plants are used in the several ways like food, medicine and of other economical and social importance. The plant *Kaempferia rotunda* Linn belongs to the family Zingiberaceae also named bhuichampaka (Sanskrit), bhuchampa (Hindi), and blackhorn (English). It is a fragrant aromatic herb with a tuberous rhizome distributed throughout India. In some districts of Maharashtra the powder root is popular in mumps and also said to be used in the form of poultice, promotes suppuration. The main constituent crotopoxide is useful

for the inhibition of tumors (Kupchun *et al.*, 1969). Phytochemical the plant has been attributed to contain flavonoids, crotepoxide, chalcones, quercetin, flavonols, β – sitosterols, stigmasterol, syringic acid, protocatechuic acid and some hydrocarbons have been previously reported (Pai *et al.*, 1970).

Materials and Methods

Plant material

The leaves of *Kaempferia rotunda* Linn were collected from local market of etawah (Uttar Pradesh). The plant was identified at Pharmacognosy department of Sir Madanlal Institute of Pharmacy, Etawah (UP), and India. The voucher specimen of *Kaempferia rotunda* Linn (kr-14) has been preserved in our herbarium for further collection and reference.

Preparation of extracts

The leaves were washed thoroughly, dried under a shade and pulverized. The coarse powder was extracted with aqueous and 95% methanol using a soxhlet apparatus. The *Kaempferia rotunda* extracts were dried using a rotary vacuum evaporator and stored in a desiccators until further use.

Phytochemical screening

The aqueous and methanolic extract of *Kaempferia rotunda* were subjected to phytochemical screening (Kokate, 1994; Khandelwal, 2004) to identify the nature of chemical constituents present in the plant material.

Animals

Wister albino rats of weighing about 150-200 gm and adult albino mice of weighing

25 -30 gm were employed for this study and were procured from institute animal house. They were fed with standard diet and water and housed in cages at room temperature ($30\pm 2^{\circ}\text{C}$) with a 12 h light and dark cycle. Ethical clearance required for the animal experiment was obtained from institute animal ethical committee (IAEC).

Acute toxicity studies (LD50)

Acute toxicity study of aqueous and methanolic extract of *Kaempferia rotunda* was performed in albino rats according to OECD guidelines 423 (OECD, 1996). The animals were kept fasting for overnight providing only water, after administration aqueous and methanolic extract orally at doses of 5– 2000 mg/kg. Animals were then allowed free access to food and water and observed a period of 48 hrs for signs of acute toxicity. The number of deaths within this period was recorded.

Wound models

The studies were carried out using ether-anesthetized rats and their back was shaved. The selected animals were divided into two groups of six in each. All the test samples were applied topically models at two different dose levels of 250 and 500 mg/kg body weight.

Incision wounds

Two Para vertebral incisions (6 cm long) were made through the full thickness of the skin on either side of the vertebral column of the rat (Lee, 1968). Wounds were closed with interrupted sutures, 1 cm apart. The sutures were removed on the seventh day. Wound-breaking strength was measured in anesthetized rats on the tenth day after wounding (Morton and Malone, 1972).

Table.1 Incision modal

Solvents	Breaking strength(g)
Control	211.333±4.780
aqueous extract of <i>Kaempferia rotunda</i> 250mg/kg	367.105±2.140
aqueous extract of <i>Kaempferia rotunda</i> 500mg/kg	327.653±1.865
Methanolic extract of <i>Kaempferia rotunda</i> 250mg/kg	415.983±1.654
methanolic extract of <i>Kaempferia rotunda</i> 500mg/kg	390.653±1.974

Table.2 Excision modal

Wound model Parameter Studies	Excision % of wound contraction		
	4 th	8 th	12 th
Control	23.577 ±0.165	33.473±0.079	53.667 ±0.123
aqueous extract of <i>Kaempferia rotunda</i> 250mg/kg	25.373 ±0.164	46.587 ±0.096	80.387 ±0.033
aqueous extract of <i>Kaempferia rotunda</i> 500mg/kg	26.480 ±0.320	52.796±0.094	83.594±0.077
methanolic extract of <i>Kaempferia rotunda</i> 250mg/kg	26.323 ±0.163	49.537 ±0.099	87.347,±0.075
methanolic extract of <i>Kaempferia rotunda</i> 500mg/kg	26.870 ±0.320	59.757±0.077	93.503 ±0.074

Figure.1 Break strength

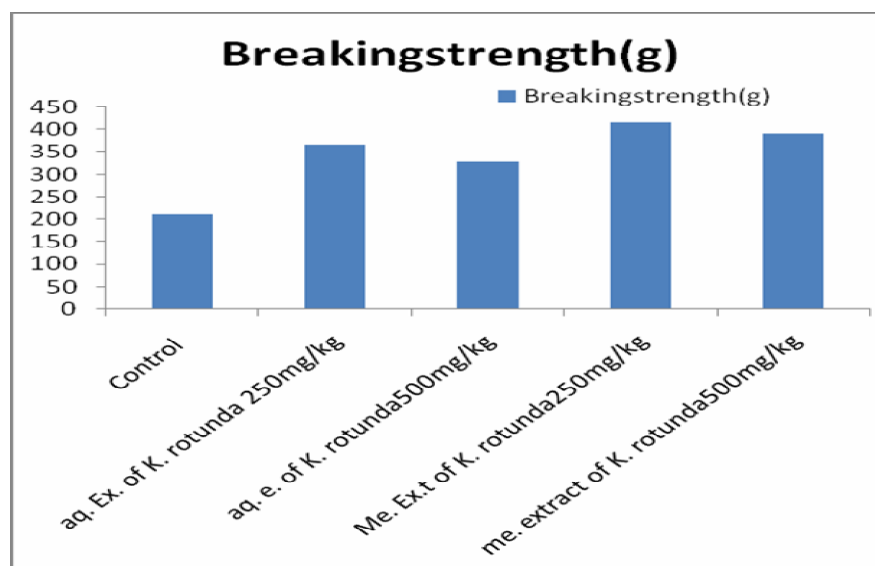
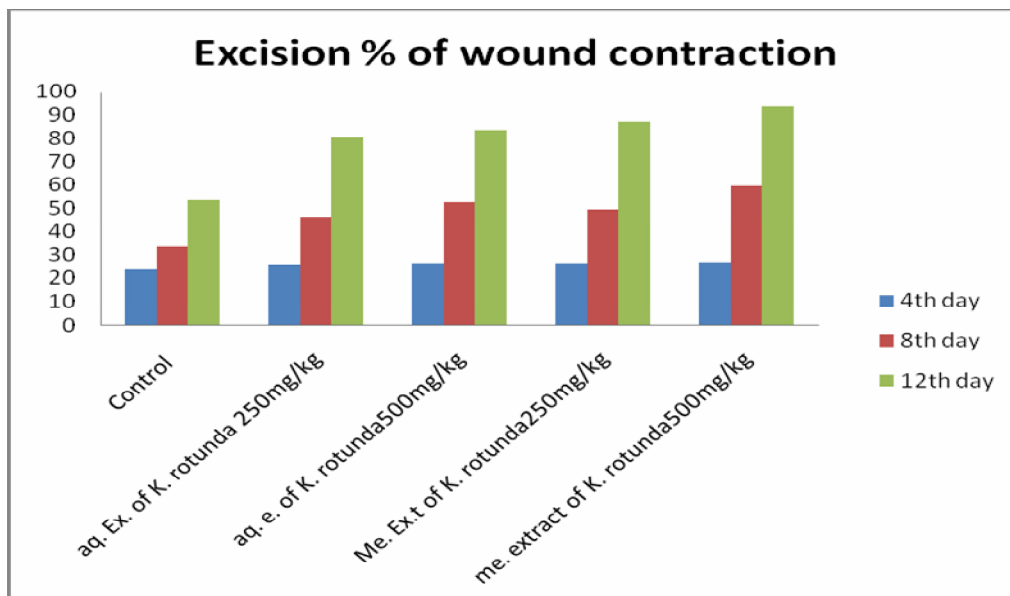


Figure.2 Excision % of wound contraction



Excision wounds

A circular skin piece of full thickness (approximately 500 mm²) was removed from a predetermined dorsal area (Neuman and Logan, 1950). The wounds were traced on 1 mm² graph paper on the day of wounding and subsequently on alternate days until healing were complete. Changes in the wound area were calculated, giving an indication of the rate of wound contraction.

Statistical analysis

All the results are expressed as mean \pm standard error. The data was analyzed statistically using ANOVA.

Result and Discussion

Phytochemical screening

The phytochemical tests revealed the presence of Flavonoids, crotepodid, chalcones, quercetin, protocatechuic acid, β -sistosterol, stigmasterol, syringic acid, and some hydrocarbons in methanol extract.

Acute toxicity test (LD50)

Oral administration of graded doses (5 to 2000 mg/kg) of the aqueous and methanolic extract of *Kaempferia rotunda* to rats did not produce any significant changes in behavior, breathing, cutaneous effects, sensory nervous system responses or gastrointestinal effects during the observation period. No mortality was recorded in any group after 72 hr of administering the extract to the animals.

Wound healing

The results of plant in wound healing property are given in the table-1 and table-2.

Wound healing process consists of different phases such as granulation, collageneration, collagen maturation and scar maturation which are concurrent but independent to each other. Hence in the present study two different wound models were used. In the incision wound model, a significant increase was observed in the skin breaking strength of the aqueous and

methanolic extract treated group at both dose levels (Table 1). The study on the excision wound animal model revealed that the drug extract affect the rate of wound contraction significantly at both dose levels (Table 2).

The above finding provides an insight into the usage of *Kaempferia rotunda* Linn leaf in traditional treatment of wounds, burns and reduces swelling.

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