Absorbance ratio method[12] depends on the property that, for a substance which obeys Beer's Law at all wavelengths, the ratio of absorbance at any two wavelengths is a constant value independent of concentration or path length. Quantitatively, absorbances are measured at two wavelengths one being the λ_{max} of PIO-H at 268.6 nm for estimation of PIO-H, and the other being a wavelength of equal absorptivity of two components i.e. isoabsorptive point 251.5 nm for estimation of GLIM. Both the methods were successfully used to estimate the amount of PIO-H and GLIM present in marketed tablet formulations containing PIO-H and GLIM. The assay values for tablets, by both the methods are in the range 99.4-99.6 % and 100.2-100.2 % for PIO and GLIM, respectively. The results obtained were comparable with the corresponding labeled amounts (Table 1).

By observing the validation parameters, accuracy, precision expressed as coefficient of variation (CV) ruggedness (interday, intraday, different analysts), specificity, linearity (correlation coefficient, $r^2<1$) and range, both the methods were found to be specific, accurate, precise, reproducible and rugged. Hence both the methods can be employed for routine analysis of tablets for assay.

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REFERENCES

1. Budavari, S., Eds., In; The Merck Index, 13th Edn., Merck and Co., Inc., Whitehouse Station, NJ, 1997, 7533.
2. Budavari, S., Eds., In; The Merck Index, 13th Edn., Merck and Co., Inc., Whitehouse Station, NJ, 1997, 4453.
3. Radhakrishna, T. and Sreenivas Rao, **J. Pharm. Biomed. Anal.,** 2002, 29, 593.
4. Jundong, D. and Wujia, J., **Yaowu Fenxi Zazhi,** 2001, 21, 36.
5. Kenji, Y. and Motohashi, M., **J. Chrom. B.: Biomed. Sci. Appl.,** 1996, 677,141.
6. Lin, Z.J., Ji, W., Desai-Kriegar, D. and Shum, L., **J. Pharm. Biomed. Anal.,** 2003, 33, 101.
7. Lehr, K.H. and Damm, P., **J. Chrom.,** 1990, 526, 497.
8. Lad, R.N., Bhoir, S.J., Bhoir, I.C. and Sundaresan, M., **Indian J. Pharm. Sci.,** 2003, 65, 650.
9. Wang, W. and Xie, J., **Yaowu Fenxi Zazhi,** 2002, 22, 474.
10. Actinoz, S. and Takeli, D., **J. Pharm. Biomed. Anal.,** 2001, 24, 507.
11. Davidson, A.G., In; Beckett, A.H. and Stenlake, J.B., In; Practical Pharmaceutical Chemistry, 4th Edn., Part II, CBS Publisher, New Delhi, 1997, 284.
12. Davidson, A.G., In; Beckett, A.H. and Stenlake, J.B., In; Practical Pharmaceutical Chemistry, 4th Edn., Part II, CBS Publisher, New Delhi, 1997, 286.

Wound Healing Activity of Leaves of *Artocarpus heterophyllus*

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The ethanol extract of dried leaves of *Artocarpus heterophyllus* Lam. and different crude fractions such as petroleum ether (40-60°), butanol, butanone and methanol were tested for various phytoconstituents and screened for wound healing properties using incision, excision and dead space (granulation) wound models in albino rats. The methanol fraction had exhibited the most significant wound healing property followed by butanol, butanone fractions and ethanol extract, where as petroleum ether fraction was least effective.

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Artocarpus heterophyllus Lam. (Fam: Urticaceae) is a large tree, evergreen 10-15 m in height, indigenous to the evergreen forest of Western Ghats at an altitude of 450-1200 m and cultivated throughout the hotter parts of India[1]. Young leaves have been reported to be used in skin diseases and have also been considered as antidote to the snake poison[2]. Leaves have also been applied on boils and wounds to dry them[3]. A review of literature however did not reveal any information on the wound healing properties of this plant. The present study is an attempt to assess the efficacy of this indigenous tree on various parameters related to wound healing in Wistar rats.

The leaves of *Artocarpus heterophyllus* were collected from the local area of Belgaum, Karnataka and were authenticated at the R. L. Sc. Institute, Belgaum. A voucher specimen (KL450) has been deposited at the K. L. E. S's College of Pharmacy, Belgaum.

The air-dried leaves of *Artocarpus heterophyllus* around 1.5 kg were reduced to a fine powder and the powder was subjected to continuous extraction with hot ethanol in 10 batches of 150 g each in a Soxhlet extractor. After the complete extraction, the solvent was distilled off and concentrated on a water bath to a dry residue. The concentrated ethanol extract was dispersed in 250 ml distilled water and subjected to fractionation with petroleum ether (40-60°), butanol, butanone and methanol in succession. Each fraction was washed with water, then dried over anhydrous sodium sulphite and concentrated and then evaporated to dryness. The individual fractions were subjected to various chemical tests to detect the presence of different phytoconstituents[4,5].

Healthy adult Wistar rats of either sex weighing 150-200 g were selected for the acute toxicity study. The study protocol was approved by the Animal Ethics Committee of the Institution (CPCSEA Registration No. 221). The animals were starved overnight, divided into 6 groups (n=2) and were fed with increasing dose (10, 30, 100, 300, 1000, 2000 mg/kg) of the ethanol extract of *A. heterophyllus* and its fractions. The animals were continuously observed for mortality and behavioural responses for 48 h and thereafter once daily for 14 d after administration.

Wound healing studies were carried out using ether-anaesthetised rats. Animals were divided into six groups of six animals each. Animals were depilated at the desired site before wounding. They were housed individually with free access to food and water, the basal food intake and body weights to the nearest gram were noted. The animals were starved for 12 h prior to the study. The first group received the vehicle (gum acacia 1%) orally and served as the control group. Second, third, fourth, fifth and sixth groups received ethanol extract, petroleum ether (40-60°), butanol, butanone and methanol fractions, respectively by oral route at a dose of 100 mg/kg daily for 10 consecutive days in the incision and dead space wound model and for 18 days in the excision wound model.

The incision wound method of Ehrlich and Hunt[6] was adopted. Under light-ether anaesthesia two paravertebral incisions of 6 cm were made through the entire thickness of the skin, on either side of the vertebral column with the help of a sharp blade. The incisions were sutured using 4-0 silk threads with the help of a straight round-bodied needle. On day 8 post wounding, sutures were removed and the breaking strength was determined 2 d later on the day 10 after infliction of the wound, using the continuous constant water flow technique reported by Lee[7].

In the excision wound method, a circular piece of full thickness (approximately 500 mm^2) skin was cut off from a predetermined area on the back of the rat[8]. The observations on percent wound closure, epithelization period and size of scar area were made on day 4, 8, 12 and 18 post-wounding.

In the dead space wound method, physical changes in the glaucoma tissue were studied. Under light-ether anaesthesia, subcutaneous dead space wounds were inflicted in the region of the axilla and groin by making a pouch through a small nick in the skin. Cylindrical grass piths measuring 2.5 cm in length and 0.3 cm in diameter were introduced into the pouch to harvest granulation tissue. Each animal received two grass piths in different locations. The wounds were sutured and mopped with an alcoholic swab. Animals were placed in to their individual cages after recovery from anaesthesia. Excision of the granuloma from the surrounding tissue was performed on the day 10 post-wounding under light ether anaesthesia. Granulation tissues surrounding the grass piths were excised and slit open. The breaking strength of piece measuring about 15 mm in length and 8 mm in width (obtained by trimming the rectangular strip of granuloma tissue) was determined on day 10 after wounding by continuous constant water flow technique of Lee[7]. All the results were analysed by Student's t-test and the level of significance was set at $P<0.001$.

The average yields of the ethanol extract and different

TABLE 1: EFFECT OF LEAVES EXTRACTS ON THE BREAKING STRENGTH OF INCISION WOUND

Group	Oral Dose (mg/kg)	Breaking strength (g)
Control	0.5 ml of 1% gum acacia	122.3±14.1
Methanol	100	213.3±12.9*
Butanone	100	200.3±19.1
Butanol	100	192.9±5.2*
Ethanol	100	196.5±11.6*
Petroleum Ether	100	181.1±12.9

All value are mean±SE, *P<0.001 Vs Control, n=6 (number of animals)

fractions of the ethanol extract of the leaves of *Artocarpus heterophyllus* Lam. were found to be 1.16% w/w, butanone- 1.55% w/w, butanol- 1.94% w/w, petroleum ether- 2.58% w/w and methanol- 2.58% w/w, respectively. Sterols, triterpenoids, glycosides, alkaloids, flavonoids, tannins, carbohydrates were found to be present in the ethanol extract and its fractions as revealed by the qualitative tests. In resutured incision wound model, ethanol extract and its fraction showed increased mean breaking strength compared to control. The maximal activity seen with the methanol fraction was 213.3±12.9 g, with the butanone fraction was 200.3±19.1 g, butanol fraction was 92±5.2 g, ethanol extract was 196.5±11.6 g and with the petroleum ether fraction was 181.1±12.9 g, which were highly significant as compared to control (Table 1).

The excision wound heals by contraction and epithelisation. The parameters studied included wound closure, time of epithelisation (days) and size of scar area (mm^2). The percent wound closure after treatment with different fractions was evaluated at different intervals (days) post wounding. The results showed that the methanol fraction promoted better wound healing (95.3%) as compared to control (86.0%) and other fractions. The results indicated that methanol fraction, butanone fraction, butanol fraction, petroleum ether fraction and the ethanol extract promoted complete epithelisation by 17.0, 17.7, 18.5, 19.5 and 19.2 days, respectively when compared to the time taken for complete epithelization of 21.5 days in the control group.

This very well signifies better wound healing activity with methanol fraction. The results also indicated least scar formation with methanol fraction of 7.2 mm^2 followed by other fractions when compared to the scar of 28.2 mm^2 in control animals (Table 2).

Similar results were obtained for breaking strength of the grass pith induced granuloma (Table 3). The results showed significant activity in case of methanol fraction (248±5.1 g) as compared to control (130±10.9 g). The present investigation revealed that methanol fraction of leaves of *A. heterophyllus* Lam. has significant wound healing activity in Wistar rats.

The results of our study showed that the methanol fraction of the ethanol extract of the leaves of *A. heterophyllus* possessed a definite prohealing action. This is demonstrated by a significant increase in the rate of wound contraction by enhanced epithelisation, increase in skin breaking strength, which is further supported by gain in granuloma breaking strength. All these findings indicate increased collagen maturation by increased cross-linking. From the results of the present investigation, it may be concluded that the plant *A. heterophyllus* is endowed with significant wound heal-

TABLE 2: EFFECT OF LEAVES EXTRACTS ON THE EXCISION WOUND PARAMETER.

Group	% Wound Closure				Epithelisation period (d)	size of scar area mm^2
	Day 4	Day 8	Day 12	Day 18		
Control	46.6±2.0	60.0±1.9	81.1±1.2	86.0±1.1	21.5±0.4	28.2±1.5
Methanol	60.1±1.7*	78.3±1.4*	91.1±1.6*	95.3±1.0*	21.5±0.4	7.2±1.2*
Butanone	64.2±1.6*	68.2±2.5	80.8±2.3	93.0±1.5	17.7±0.8*	7.2±1.2*
Butanol	64.2±1.7*	76.0±3.7	89.6±2.1	94.7±1.5*	18.5±0.8*	11.2±2.2*
Ethanol	59.0±1.7*	68.5±1.6	86.4±1.7	92.5±1.9	19.2±0.3*	19.7±2.7
PetroleumEther	65.5±2.2*	68.2±2.6	81.9±2.1	86.6±1.9	19.5±0.6	22.3±1.7

All value are mean ± SE *P<0.001 Vs Control, n = 6 (n = number of animals)

TABLE 3: EFFECT OF LEAVES EXTRACTS ON BREAKING STRENGTH OF THE GRASS PITH INDUCED GRANULOMA STUDIES.

Group	Oral Dose (mg/kg)	Breaking strength (g)
Control	0.5 ml of 1% Gum acacia	129.8±10.9
Methanol	100	248.0±5.1*
Butanone	100	135.0±11.9
Butanol	100	210.8±6.6*
Ethanol	100	165.0±12.0
Petroleum ether	100	161.0 ±10.9

All value are mean±SE, *P<0.001 Vs Control, n = 6 (number of animals)

ing activity, there by justifying its use in the indigenous system of medicine.

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REFERENCES

1. Wealth of India, a Dictionary of Indian Raw Material and Industrial Products, Vol. I A, Publications and Information Directorate, CSIR, New Delhi, 1948, 447.
2. Nadkarni, K.M., In; Indian Materia Medica, 3rd Edn., Vol. I, Popular Prakashan, Mumbai, 1976, 147.
3. Basu, B.D. and Kirtikar, K.R., Indian Medicinal Plants, 2nd Edn., Vol. III, Lalit Mohan Basu, Allahabad, 1935, 2337.
4. Harbone, J.B., In; Phytochemical Methods, Chapman and Hall, London, 1998, 60.
5. Kokate, C.K., In; Practical Pharmacognosy, 3rd Edn., Vallabh Prakashan, New Delhi, 1994, 107.
6. Ehrlich, H.P. and Hunt, T.K. **Ann. Surg.**, 1969, 170, 203.
7. Lee, K.H., **J. Pharm. Sci.**, 1968, 57, 1042.
8. Morton, J.J.P. and Malone, M.H., **Arch. Int. Pharmacodyn.**, 1972, 196, 117.

A Comparative Dissolution Study of Commercial and Prepared Formulations of Celecoxib

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Celecoxib, a COX-2 inhibitor, useful in the relief of symptoms of rheumatoid arthritis, suffers from the draw back of poor aqueous solubility, thereby giving problems during formulations and in achieving good oral bioavailability. The present paper attempts at preparing three formulations of celecoxib in conjunction with β-cyclodextrin for the purpose of solubility enhancement of the drug. The formulations were tested in different media (water, 0.1 N HCl, phosphate buffer pH 7.4). Phosphate buffer was found to be the most suitable amongst the three with good discriminating power. The formulations were compared with two marketed capsule samples of celecoxib. A marked enhancement in the dissolution of celecoxib from the laboratory made formulations was observed as compared to the marketed preparations.

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