THIN LAYER CHROMATOGRAPHIC STUDIES AND ASSESSMENT OF ANTI-INFLAMMATORY EFFECT OF *HIBISCUS SCHIZOPETALUS* LEAF EXTRACTS IN RATS

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Summary

Hibiscus schizopetalus (Dyer) Hook.f. (Malvaceae), commonly known as *Jhumko Jaba* in Bengali, Japanese Lantern and Coral Hibiscus in English, is a shrub indigenous to tropical Eastern Africa in Kenya, Tanzania and Mozambique and also grown in the Indian subcontinent. The present study assessed the different solvent extracts of *H. schizopetalus* leaf for thin layer chromatography (TLC) and also evaluated their acute anti-inflammatory potential by carrageenan induced paw oedema in Wistar albino rats. The chloroform extract yielded maximum numbers of spots in TLC, followed by methanol and petroleum ether extracts respectively. All of the test extracts exhibited significant anti-inflammatory activity. The methanol extract was found to be the most potent followed by the chloroform and petroleum ether extracts respectively. The present preliminary study demonstrated marked acute anti-inflammatory activity of *H. schizopetalus* leaf in Wistar rats.

Key words: *Hibiscus schizopetalus,* anti-inflammatory, oedema, leaf.

Introduction

Hibiscus schizopetalus (Dyer) Hook.f. (Malvaceae), commonly known as *Jhumko Jaba* in Bengali, Japanese Lantern and Coral Hibiscus in English, is a shrub with distinctive red or pink flowers indigenous to tropical Eastern Africa in Kenya, Tanzania and Mozambique and also grown in subtropics, like the Indian subcontinent. It has highly decorative hanging flowers with frilly, finely dissected petal have a range of colours, the most common being the red form (1, 2).

Previous researchers reported the major anthocyanin found in flowers of *H. schizopetalus* is cyanidin-3-sambusophoroside (3). From its leaves, two new triterpene esters have been isolated (4). Previous workers have also reported antioxidant, anti-tyrosinase and antibacterial activities of *H. schizopetalus* (5). However, the planar chromatographic profile and anti-inflammatory assessment of petroleum ether, chloroform and methanol extracts from *H. schizopetalus* leaf are still not reported. Therefore, in the present investigation we attempted these studies on the different leaf extracts of *H. schizopetalus* grown in India.

Materials and methods

Plant material: The mature leaves of *Hibiscus schizopetalus* (Dyer) Hook.f. (Malvaceae), were collected during June 2011 from Halisahar region of 24-Parganas district of West Bengal, India. The plant material was taxonomically identified at the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India. The voucher specimen [CNH/68/2011/Tech II/548] was maintained in our research laboratory for future reference. The plant material was shade-dried with occasional shifting and then powdered with mechanical grinder, passing through sieve no. 40, and stored in an air-tight container.

Preparation of plant extracts: The dried powdered material (350 g) was defatted with petroleum ether (60-80°C), the percentage extractive value was 1.02% w/w. The defatted powder material thus obtained was further extracted with chloroform and methanol for 72 h in a percolator. The solvent was distilled off in reduced pressure and resulting semisolid mass was vacuum dried using rotary flash evaporator to yield a solid residue and the percentage extractive values were accordingly 3.27% w/w and 13.97% w/w respectively. The preliminary phytochemical analysis was performed for all three extracts to identify the phytoconstituents present in the extracts (6).

Drugs and chemicals: λ-Carrageenan (type IV) was obtained from S. D. Fine Chemicals Ltd., Bombay; indomethacin was from Recon, Bangalore, India. All other chemicals and reagents were of analytical grade obtained commercially.

Experimental animals: Studies were carried out using adult male Wistar albino rats of weighing 150-180 g. The animals were grouped in polyacrylic cages (38 cm × 23 cm × 10 cm) with not more than four animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2 °C, dark and light cycle 14/10 h). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The rats were acclimatized to laboratory condition for 10 days before commencement of experiment. All experimental methods were reviewed and approved by the Institutional Animal Ethical Committee.

Thin layer chromatographic studies: Each solvent extract was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel G as stationary phase. The mobile phases, results and chromatograms are depicted in Figures 1-3.

Evaluation of anti-inflammatory activity

***Carrageenan-induced rat paw oedema*:** The rats were divided into five groups (n = 6). The first group (which served as control) received normal saline (3 ml/kg body wt., p.o.). The second, third and fourth group received the three test extracts (200 mg/kg body wt., p.o., each). The fifth group (which served as reference) received indomethacin (10 mg/kg body wt., p.o.). After 30 mins, acute inflammation was produced by the sub-plantar administration of 0.1 ml of 1 % (w/v) of freshly prepared suspension of carrageenan in the right hind paw of each rat. The paw volume was measured at 0 hour and at each hours up to 4 hours after carrageenan injection by using plethysmometer (Ugo Basile, Italy). The difference between the two readings was taken as the volume of oedema and the percentage of inhibition was calculated by using the following formula (7, 8).

(Control mean− Treated mean/ Control mean) × 100 %.

Statistical analysis: The values were expressed as mean ± standard error of mean (SEM). Statistical significance was analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's *post hoc* test of significance. Values of $p < 0.001$ were considered as statistically significant.

Results and discussion

Preliminary phytochemical studies on *H. schizopetalus* leaf extracts showed the presence of triterpenoids and steroids in the petroleum ether extract; triterpenoids and steroids in the chloroform extract; and glycosides, phenolic compounds, tannins, carbohydrates in the methanol extract.

Among the various methods for separating plant constituents, the thin layer chromatographic procedure is the one of the most commonly used techniques of general application (9). Thin layer chromatography (TLC) involves the separation of mixtures of organic compounds on thin layers of adsorbents that are usually coated on glass, plastic, or aluminum sheets; and this particular technique is the easiest, cheapest and most widely used method for the characterization of natural products and their preparations (10). The chloroform extract yielded maximum numbers of spots in TLC, followed by methanol and petroleum ether extracts respectively (Figures 1-3). All of these TLC profiles may serve as characteristic fingerprint of *P. guajava* leaf. These data would therefore be suitable for monitoring the identity and purity of the plant material and for detecting adulterations and substitutions (11).

Anti-inflammatory activity of *H. schizopetalus* leaf extract was evaluated against carrageenan induced acute paw oedema in rats and the results are summarized in Table 1. The methanol extract was found to be the most potent followed by the chloroform and petroleum ether extracts respectively after 4 hours of treatment, whereas the reference drug indomethacin was found to be the most potent when compared with the saline control group.

The present study establishes the significant anti-inflammatory activity of *H. schizopetalus* leaf against the experimentally induced acute inflammation in rodents. Carrageenan-induced paw oedema has been commonly used as an experimental animal model for acute inflammation and it is believed to be a biphasic response. The early phase (1 - 2 h) of the carrageenan model is mainly mediated by histamine and serotonin (5-HT). The late phase (2 - 4 h) is mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins

produced by tissue macrophages (12). All the test extracts produced significant ($p < 0.001$) inhibition of carrageenan-induced rat paw oedema after a period of 4 h.

Based on the results obtained from the present preliminary investigation, it can be inferred that all the test extracts from *H. schizopetalus* leaf possessed remarkable acute anti-inflammatory property in the Wistar rats. The present preliminary study confirms marked anti-inflammatory activity of *Hibiscus schizopetalus* leaf which may be due to presence of multitude of constituents as revealed by its TLC profile.

Table 1. Effect of *H. schizopetalus* leaf extracts on carrageenan induced rat paw oedema.

Treatment	1 h	2 h	3 h	4 h	% Inhibition
Normal control	0.73±0.08	1.40±0.57	1.80±0.57	1.66±0.08	-
Indomethacin (10 mg/kg)	0.20±0.05*	0.50±0.05*	0.36±0.03*	0.23±0.03*	86.14
Pet ether extract (200 mg/kg)	0.34±0.05*	0.59±0.03*	0.46±0.05*	0.38±0.03*	77.11
$CHCl_3$ extract (200 mg/kg)	0.30±0.06*	0.52±0.07*	0.40±0.04*	0.31±0.06*	81.32
MeOH extract (200 mg/kg)	0.27±0.04*	0.45±0.06*	0.32±0.04*	0.24±0.05*	85.54

Values are mean ± SEM (n = 6). *$p < 0.001$ when compared to normal control.

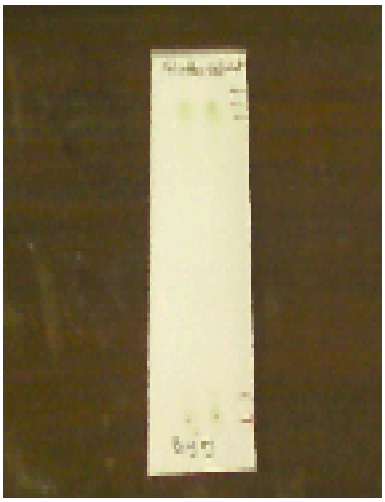

Fig. 1. TLC profile of the pet. ether extract of *H. schizopetalus* leaf. Solvent system: benzene: chloroform: ethyl acetate (4: 3: 3). R_f values: 0.06, 0.91, 0.95.

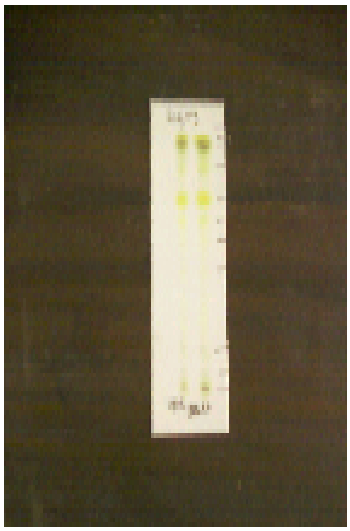

Fig. 2. TLC profile of the chloroform extract of *H. schizopetalus* leaf. Solvent system: benzene: chloroform: ethyl acetate (3: 4: 3). R_f values: 0.06, 0.15, 0.44, 0.56, 0.64, 0.72, 0.83, 0.94, 0.96.

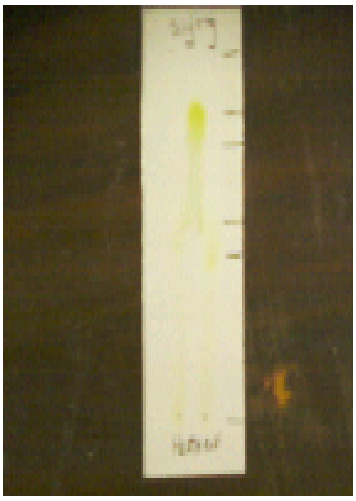

Fig. 3. TLC profile of the methanol extract of *H. schizopetalus* leaf. Solvent system: ethyl acetate: methanol (7: 3). R_f values: 0.44, 0.53, 0.74, 0.82.

References

1. Ng, F.S.P. 2006. Tropical Horticulture and Gardening. Clearwater Publications, Kuala Lumpur, Malaysia. pp. 361.
2. Dasuki, U.A. 2001. Hibiscus. In van Valkenburg, J.L.C.H. and Bunyapraphatsara, N. (eds.). Plant Resources of South-East Asia No. 12(2): Medicinal and Poisonous Plants 2., pp. 297-303. Backhuys Publisher, Leiden, Netherlands.
3. Lowry, J.B. (1976). Floral anthocyanins of some Malaysian *Hibiscus* species. Phytochemistry 15: 1395-1396.
4. Jose, E.A., Vijayan, K.K. (2006). New taraxerane esters from Hibiscus schizopetalus leaves. Indian Journal of Chemistry - Section B, Organic and Medicinal Chemistry 45 (5): 1328-1331.
5. Wong S.K., Y.Y. Lim, E.W.C. Chan. Evaluation of antioxidant, anti-tyrosinase and antibacterial activities of selected *Hibiscus* species. Ethnobotanical Leaflets 14: 781-96. 2010.
6. Kokate CK (1994): Practical Pharmacognosy. 4th Edition. New Delhi. Vallabh Prakashan. pp. 107-112.
7. Winter CA, Risley EA, Nuss, GW. Carrageenan-induced oedema in hind paw of the rats as assay for antiinflammatroy drugs. Exp Biol Med 1962; 111: 544-547.
8. Bhattacharya S, Haldar PK, Zaman MK. Anti-inflammatory and *in vitro* antioxidant property of *Zanthoxylum nitidum* root. Curr Trends Biotech Pharm 2010; 4: 774-783.
9. Kokate C.K., Purohit A.P., Gokhale S.B. Pharmacognosy. 34th ed. Nirali Prakashan: Pune, 2006.
10. Stahl E. Apparatus and general techniques. In: Stahl E., editor. Thin Layer Chromatography, A Laboratory Handbook. 2nd ed. Springer-Verlag: Berlin, Heidelberg, New York, 1969, 52-85.
11. Ghosh A.K., Bhattacharya S. Planar chromatographic studies on *Abies webbiana* leaves. Int J Chem. Tech. Res. 2009; 1: 807-814.
12. Brito ARMS, Antonio MA. Oral anti-inflammatory and antiulcerogenic activities of a hydroalcoholic extract and partitioned fractions of *Turnera ulmifolia* (Turneraceae). J Ethnopharmacol 1998; 61: 215-228.