The Asian and Australasian Journal of Plant Science and Biotechnology ©2010 Global Science Books

Pharmacognostic Evaluation of the Leaves of *Kirganelia reticulata* Baill. (Euphorbiaceae)

S. D. Shruthi[1] • Y. L. Ramachandra[1*] • S. Padmalatha Rai[2] • Prashant Kumar Jha[3]

[1] P.G. Department of Studies and Research in Biotechnology & Bioinformatics, Kuvempu University, Shankaraghatta – 577 451, Karnataka, India
[2] Department of Biotechnology, Manipal Life Science Center, Manipal University, Manipal-576104, India
[3] Quality Control Laboratories, ALN Rao Memorial Medical College, Koppa, Chikmagalur-577126, Karnataka, India

Corresponding author: * **ylrpub@gmail.com**

ABSTRACT

The leaves of *Kirganelia reticulata* Baill. (Euphorbiaceae) are known to have many uses in Indian ethnomedicine. Establishment of a pharmacognostic profile of the leaves will assist in standardization for quality, purity and sample identification. Evaluation of the fresh, powdered and anatomical sections of the leaves were carried out to determine the macro- and micromorphological characters, and quantitative, qualitative and phytochemical profiles. The results of the study could be useful in setting some diagnostic indices for the identification and preparation of a monograph of the plant.

Keywords: fluorescence, pharmacognosy, physicochemical, phytochemical studies, quantitative leaf microscopy

INTRODUCTION

After decades of serious obsession with the modern medicinal system, people have started looking at the ancient healing systems like Ayurveda, Siddha and Unani. This is because of the adverse effects associated with synthetic drugs (Thomas *et al*. 2008). Plants have been associated with the health of mankind from time immemorial. In the past sickness was viewed as a punishment from the god's and hence was treated with prayers and rituals which included "magic proportion" prepared from local herbs (Sandhya *et al*. 2010). Herbal drugs play an important role in health care programs especially in developing countries. However a key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries, is the lack of documentation of research work carried out on traditional medicines and stringent quality control (Dahanukar *et al*. 2000). With this backdrop, it becomes extremely important to make an effort towards standardization of the plant material to be used as medicine.

Kirganelia reticulata (Synonym: *Phyllanthus reticulatus*; Bengali name: Panjuli; Family- Euphorbiaceae) is a large, often scandent, shrub. The plant grows throughout tropical areas of India, Bangladesh, China, and the Malay Islands (Kirtikar and Basu 1980; Ghani 2003). The biological work performed so far on this plant showed hypotensive effects and its folkloric use in gastric complaints including colic, constipation etc. and chemical studies demonstrated the presence of octacosanol, teraxerol acetate, friedeline, teraxerone, betulin, sitosterol etc. (Rav *et al.* 1964; Joshi *et al.* 1991). The leaves are employed as a diuretic and cooling medicine. The juice of the leaves is used to care diarrhoea in infants. The stems are used to treat sore in eyes and the powdered leaf is used in sores, burns, suppurations and chafing of the skin (Chopra *et al.* 1956). The bark is used to treat rheumatism, dysentery and venereal diseases (Yoganarasimhan 1996). The plant is used for a variety of ailments, including smallpox, syphilis, asthma, diarrhoea, bleeding from gums (Nandkarni 1982; The Wealth of India 2005). It is also claimed to have antidiabetic activity in tribal areas, which has been validated by Kumar *et al*. (2008). The antibacterial potential of the leaf extracts of this plant has been evaluated recently (Shruthi *et al*. 2010). We now report on the pharmacognostic profile and confirm the ethnopharmacological claim of the plant.

Herbs show a number of problems when quality aspect is considered. This is because of nature of the herbal ingredients and different secondary metabolites present therein. It is also due to variation in the chemical profile of herbs due to intrinsic and extrinsic factors like growth, harvesting, geographical source, storage and drying etc (WHO 2002). To ensure reproducible quality of herbal medicines, proper control of starting material is utmost essential, the first step towards it is authentication followed by creating numerical values of standards for comparison (Agarwal 2005). Some drugs of plant origin in conventional medical practice are not pure compounds but direct extracts or plant materials that have been suitably prepared and standardized (Donald 1986). The World Health Organisation (WHO) has recommended the use of arthemisinin derivatives from *Artemisia annua* (Composite), a Chinese herb with established pharmacognostic data, as a first line drug in the treatment of malaria (WHO 2001, 2002) and identification of samples.

Pharmacognostical parameters like macroscopy, quantitative leaf microscopy, fluorescence, physicochemical and phytochemical studies are few of the basic protocol for standardization of herbals. Hence, in the present work establishment of the pharmacognostic profile of the leaves of *K. reticulata* is carried out; which will assist in standardization, can guarantee quality, purity and it can also be used to prepare a monograph for the proper identification of the plant.

MATERIALS AND METHODS

Fresh plant materials were collected in winter season locally from Bhadra Wild Life Sanctuary, Karnataka (Southern India) in December 2009. The taxonomic identification of the plant was confirmed by Dr. Y. L. Ramachandra, Department of Biotechnology, Kuvempu University, Shankaraghatta (Voucher specimen number YLR429).

Received: 28 July, 2010. Accepted: 21 August, 2010.

Original Research Paper

Macroscopy

The following macroscopic characters for the fresh leaves were noted: size and shape, colour, surfaces, venation, presence or absence of petiole, the apex, margin, base, lamina, texture, odour and taste (Wallis 1985; Evans 1996).

Microscopy

The outer epidermal membranous layer (in fragments) were cleared in chloral hydrate, mounted with glycerin and observed under a compound microscope. The presence/absence of the following was observed: epidermal cells, stomata (type and distribution) and epidermal hairs (types of trichomes and distribution). The transverse sections of the fresh leaves through the lamina and the midrib were also cleared, mounted and observed (African Pharmacopoeia 1986).

Quantitative investigation

Quantitative leaf microscopy to determine palisade ratio, stomata number, stomata index, vein – islet number and veinlet termination number were carried out on epidermal strips. Other physicochemical parameters determined for the powdered leaves were moisture content, total ash, acid – insoluble ash, water – soluble ash, alcohol and water-soluble extractive values (British Pharmacopoeia 1980).

Fluorescence analysis

Powdered leaf material was subjected to analysis under ultra violet light after treatment with various chemical and organic reagents like alcohol, 50% sulphuric acid, 10% sodium hydroxide, 50% nitric acid and water (Kokate 1994).

Phytochemical investigation

Chemical tests were employed in the preliminary phytochemical screening for various secondary metabolites such as carbohydrates, alkaloids, phytosterols, glycosides, saponins, flavonoids, proteins, tannins and gum (Brain and Turner 1975; Ciulei 1981; Harborne 1992; Evans 1996).

RESULTS AND DISCUSSION

Morphological characteristics of the leaf

Leaves have alternate arrangement, small or moderate sized, distichious, thin, stipulus and lanceolate (Gamble 1921). Leaves are 2.5-5 cm long and 0.7- 1.5 cm broad, coriaceous, oblong and elliptic in shape. The apex of leaves is acute with acute or subcordate base. The ventral side of leaves are dark-green in colour while dorsal side being light-green. 6-8 pairs of nerves are prominently raised on dorsal side. Leaves are pubescent along the veins otherwise glabrous. The margin of leaves is emarginated to undulated (**Fig. 1**). It is having pungent odour and has a characteristic bitter taste.

Microscopic characteristics of the leaf

The outline of transverse section is dorsiventral type. The detailed transverse section shows epidermis (both upper and lower) cover the section both in lamina and midrib portion. The upper epidermis is covered with cuticle especially in lamina portion. The lamina portion exposes that palisade cells, covers half to one third portion of lamina. The bundles of vascular are well developed and exposed in midrib portion while those of primitive type are spreaded over the lamina portion, too. The surface preparation shows the anomocytic type of stomata. The trichomes are scarcely found on the top of section along the midrib. The epidermis (both upper and lower) is followed by hypodermis, composed of collenchymatous cells in midrib. In lamina portion, the palisade parenchyma cells are in continuation with upper epidermis from above to downward while spongy parenchyma cells are followed by lower epidermis from downward to upward. These mesophyll cells (Palisade and spongy parenchyma cells) are filled with chlorophylls and at places are interrupted with vascular cells and secretory cells. In midrib portion below the hypodermis, ground tissue is present. Clusters of calcium oxalate crystals were found in parenchyma cells of this region. Vascular bundles are found in between the ground cells where xylem elements are followed by phloem elements. The vascular bundles are capped with fibres of sclerenchyma (**Fig. 2**).

The quantitative determination of some pharmacognostic parameters is useful for setting standards for crude drugs. The palisade ratio, stomata number, vein islet, vein termination numbers and the other parameters determined in the quantitative microscopy (**Table 1**), are relatively constant for plants and can be used to differentiate closely related species. The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The moisture content of the drug is not too high, thus it could discourage bacterial, fungi or yeast growth, as the general requirement for moisture content in crude drug is not more than 14% (African Pharmacopoeia 1986). Equally important in the evaluation of crude drugs, is the ash value and acid-insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign

Fig. 1 Morphology of *Kirganelia reticulata* plant along with adaxial and abaxial surfaces of the leaf.

Table 1 Quantitative leaf microscopy of *Kirganelia reticulata*.

Parameter	Range	Mean*
Palisade ratio	9-13	11.26 ± 0.42
Stomata number Upper surface	0	0
Stomata number Lower surface	59-84	72.58 ± 2.76
Stomata index Upper surface	0	0
Stomata index Lower surface	26.2-35.7	32.23 ± 1.21
Vein islet number	20-25	22.67 ± 0.52
Veinlet termination number	35-39	37.14 ± 0.39

* Mean value of 10 counts

Table 2 Fluorescence behaviour of *Kirganelia reticulata.*

Treatment	Daylight	UV light
Powder (P) as such	Pale green	Fluorescent yellowish green
P + water	Brownish green	Fluorescent orangish green
P + Alcohol[1]	Olive green	Fluorescent orange
P + 10% NaOH [2]	Blood red	Fluorescent red
P + 50% HNO_3 [3]	Orangish yellow	Fluorescent green
P + 50% H_2SO_4 [4]	Orangish green	Greenish brown

[1] Absolute alcohol;
[2] 85%, s.d. Fine Chem. Ltd., Mumbai;
[3] 69-70%, Qualigens fine chemicals, Mumbai;
[4] 97-99%, Merck Specialities Pvt. Ltd., Mumbai.

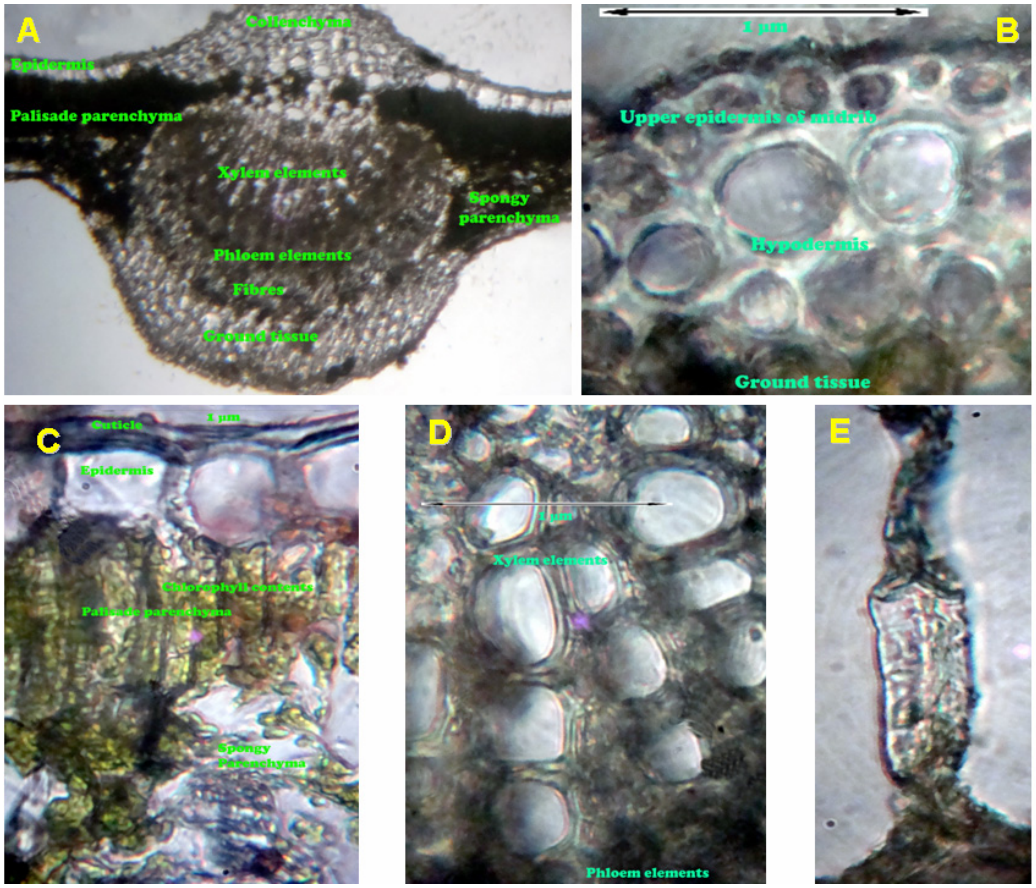

Fig. 2 Microscopy of *Kirganelia reticulata* leaf. (**A**) Outline of T.S. through midrib; (**B**) Upper epidermis of midrib and hypodermis; (**C**) Lamina portion of leaf; (**D**) Single vascular bundle showing xylem and phloem elements; (**E**) Trichome on upper surface of the leaf along the midrib.

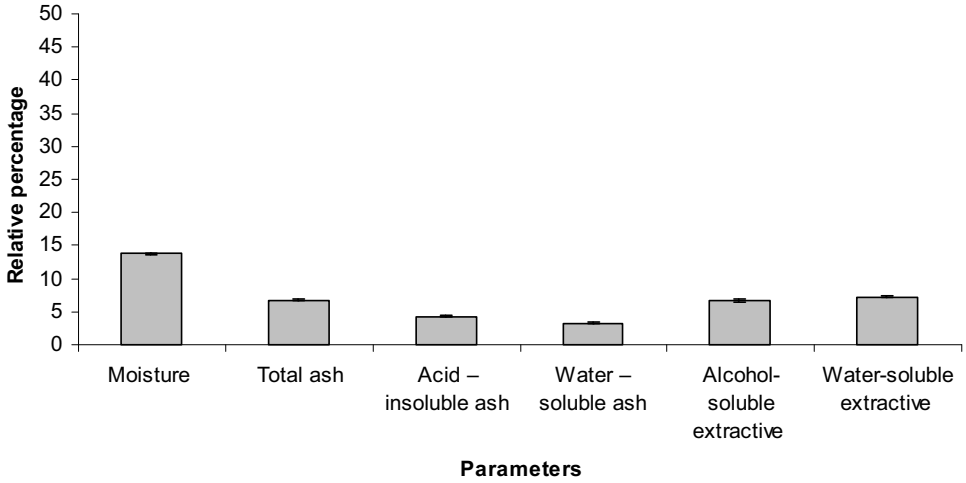

Fig. 3 Physicochemical parameters of *Kirganelia reticulata*.

inorganic matter such as metallic salts and/or silica (**Fig. 3**). The fluorescence analysis observed in day/visible light and UV light when treated with different chemical reagents, is depicted in **Table 2**. Different chemical compounds such as alkaloids, tannins, flavonoids, phytosterols, glycosides among others were detected, which could make the plant useful for treating different ailments and having a potential of providing useful drugs of human use. By virtue of their photosynthetic machinery, leaves serve as a sink for several metabolites and as an important source of several bioactive compounds (Sujan *et al*. 2009; Murti *et al*. 2010).

Empirical knowledge about medicinal plants plays a vital role in primary health care and has great potential for the discovery of new herbal drugs. These findings may be useful to supplement existing information with regard to the identification and standardization of *K. reticulata*, even in the powdered form of the plant drug, to distinguish it from substitutes and adulterants. These studies also suggested that the observed pharmacognostic and physiochemical parameters are of great value in quality control and formulation development. In conclusion, the present study may be useful to supplement information with regard to its identification and standardization, and in carrying out further research and revalidation of its use in the Ayurvedic System of Medicine.

ACKNOWLEDGEMENTS

The laboratory facility of ALN Rao Memorial Medical College, Koppa, Chikmagalur is gratefully acknowledged.

REFERENCES

African Pharmacopoeia (1986) *General Methods for Analysis*. OAU / STRC Scientific Publications, Lagos, **2 (2)**, pp 1-5, 137-149, 223-237

Agarwal A (2005) Critical issues in quality control of natural products. *Pharma Times* **37 (6)**, 9-11

Brain KR, Turner TD (1975) Practical Evaluation of Phytopharmaceuticals, Wright–Scientechnica, Bristol, 144 pp

British Pharmacopoeia (1980) *Appendix XI*, Her Majesty's Stationery Office, London, A108, **11**, A113

Chopra RN, Nayar SL, Chopra IC (1956) *Glossary of Indian Medicinal Plants* (2nd Edn), CSIR, New Delhi, India

Ciulei I (1981) *Methodology for Analysis of Vegetable Drugs*, United Nations Industrial Development Organisation, Romania, pp 17-25

Dahanukar SA, Kulkarni RA, Rege NN (2000) Pharmacology of medicinal plants and natural products. *Indian Journal of Pharmacology* **32**, 81-118

Donald EC (1986) Medicinal plant research in Nigeria: Retrospect and prospect. In: Sofowora XYZ (Ed) *The State of Medicinal Plants Research in Nigeria*, Nigerian Society of Pharmacognosy, Ibadan University Press, Nigeria, pp 1-12

Evans WC (1996) *Trease and Evans Pharmacognosy* (14th Edn), WB Saunders Ltd., London, pp 119-159

Gamble JS (1921) *Flora of the Presidency of Madras* (Vol II), Published under the authority of Secretary of State for India in Council, 1294 pp

Ghani A (2003) *Medicinal Plants of Bangladesh, Chemical Constituents and Uses* (2nd Edn), Asiatic Society of Bangladesh, 345 pp

Harborne JB (1992) *Phytochemical Methods. A Guide to Modern Technique of Plant Analysis*, Chapman and Hall, London, 279 pp

Joshi KC, Singh P, Mehta A (1991) Crystalline components of the roots of *Phyllanthus reticulatus*. *Journal of the Indian Chemical Society* **58**, 102

Kirtikar KR, Basu BD (1980) *Indian Medicinal Plants* (Vol 1, 2nd Edn), B. Singh and M.P. Singh Publishers, India, 345 pp

Kokate CK (1994) *Textbook of Pharmacognosy* (4th Edn), Vallabh Prakashan, New Delhi, pp 112-120

Kumar S, Kumar D, Deshmukh RR, Lokhande PD, More SN, Rangari VD (2008) Antidiabetic potential of *Phyllanthus reticulatus* in alloxan-induced diabetic mice. *Fitoterapia* **79**, 21-23

Murti Y, Yogi B, Pathak D (2010) Pharmacognostic standardization of leaves of *Calotropis procera* (Ait.) R. Br. (Asclepiadaceae). *International Journal of Ayurveda Research* **1**, 1

Nandkarni KM (1982) *Indian Materia Medica* (Vol 2), Publisher, City, 948 pp

Rav MRR, Siddiqui HH (1964) Screening of Indian plants for biological activity. *Indian Journal of Experimental Biology* **2**, 49

Sandhya S, Jaffery SAH, Vinod KR (2010) Pharmacognostical studies on the leaf and root of *Physalis angulatal*. *International Journal of Pharma Research and Development* **2 (1)**, 1-8

Shruthi SD, Ramachandra YL, Padmalatha Rai S, Veena Shetty A (2010) Antibacterial potential of leaf extracts from *Kirganelia reticulata* Baill. *International Journal of Pharma Research and Development* **2 (6)**, 1-7

Sujan Ganapathy PS, Ramachandra YL, Sudeep HV, Pavan Kumar Bellamakondi, Somashekhara Achar KG, Padmalatha Rai S (2009) Pharmacognostic and phytochemical evaluation of *Holarrhena antidysenterica* Wall. *The Asian and Australasian Journal of Plant Science and Biotechnology* **3**, 47-50

The Wealth of India (2005) *National Institute of Science Communication and Information Resources* (Vol 7), Council of Scientific and Industrial Research, New Delhi, 34 pp

Thomas S, Patil DA, Patil AG, Naresh Chandra (2008) Pharmacognostic evaluation and physicochemical analysis of *Averrhoa carambola* L. fruit. *Journal of Herbal Medicine and Toxicology* **2 (2)**, 51-54

Wallis TE (1985) *Textbook of Pharmacognosy*, CBS Publishers, Delhi, India, pp 572-575

WHO (2001) *Antimalarials Drug Combination Therapy*, Report of a WHO Technical Consultation, pp 1-2

WHO (2002) *Traditional Medicine Strategy 2002-2005*, World Health Organization, Geneva

WHO (2002) *WHO urges Countries to Act on New Anti-Resistance Malaria Medicines*. Press release WHO/31, pp 1-3

Yoganarasimhan SN (1996) *Medicinal Plants of India* (Vol 1), Interline Publishing Pvt. Ltd., Bangalore, Karnataka, 275 pp