

DIFFERENTIAL ELEMENTAL CHARACTERIZATION OF *HOLOPTELEA INTEGRIFOLIA*, A PREDICTIVE MEDICAMENT FOR TENNIS ELBOW.

Biochemistry

Bhagyajyothi M. Bhat	Assistant Professor, Department of Biochemistry, Kanachur Institute of Medical Sciences, Mangaluru.
Sreenivasan KK	Professor and Head, Dept. of Chemistry, Sri Madhwa Vadiraja Institute of Technology and Management, Bantakal, Udupi.
Santanu Saha*	Assistant Professor, Department of Pharmacognosy, NGSM Institute of Pharmaceutical Sciences, Deralakatte, Mangaluru *Corresponding Author

ABSTRACT

The leaves of *Holoptelea integrifolia* (*H. integrifolia*) are suggestive of having analgesic and anti-inflammatory properties, thus useful in the treatment of tennis elbow. An elementary investigation of the leaf extract was conducted in the present study as the beginning step in understanding the active ingredients present and correlating them with the suggestive role in curing different ailments. Ethanolic extract of leaves of *H. integrifolia* and successive fractionation was done with petroleum ether, chloroform, ethyl acetate, n-butanol and methanol. Phytochemical analysis of ethanolic and ethyl acetate extracts showed the presence of alkaloids, triterpenoids, saponins, steroids and tannins. Chloroform and n-butanol extracts showed alkaloids, triterpenoids, saponins and steroids. Triterpenoids, saponins, steroids and tannins are found in methanolic whereas triterpenoids and steroids in petroleum ether extract. The anti-inflammatory and analgesic activities of alkaloids, triterpenoids, and tannins may be responsible for the predictive role of *H. integrifolia* in curing tennis elbow.

KEYWORDS

Holoptelea integrifolia, preliminary phytochemical screening, alkaloids, triterpenoids.

INTRODUCTION:

Herbal medicines are being used in the treatment of various ailments since time immemorial in our country. In spite of many research activities about different phytomedicines, there are still many plants with promising medicinal effect, a detailed study of which has not been met with. One such plant is *Holoptelea integrifolia* (*H. integrifolia*), a medium sized tree of family Ulmaceae.

H. integrifolia is traditionally used in treating obesity, oedema and bronchitis. Various extracts of this plant showed different healing properties such as anti-inflammatory, anthelmintic, antibacterial, antidiarrheal, antitumour, antidiabetic, anti oxidant and wound healing properties (1). The paste made from the seeds was found to be useful in treating eczema and other cutaneous infections. Paste of the bark and the leaves is said to be useful in treatment of leucoderma (2). Ethanolic extract of *H. integrifolia* leaves was found to reduce paw oedema in experimental rats suggesting its anti-inflammatory and antitumour activities (3,4). A dose dependent inhibitory activity on paw edema in rats fed an aqueous extract of leaves of this plant at dose 250 and 500mg/kg and the percent inhibition was comparable with standard drug indomethacin (100mg/kg) (5). Analgesic activity of various extracts of *H. integrifolia* is also documented (6). Ethanolic leaf extract showed maximum activity followed by ethyl acetate, n-butanol and aqueous extracts. We received information about the beneficial effect of the crushed leaves of *H. integrifolia* in curing tennis elbow by an ayurvedic pandith though could not get relevant data for proof.

As a primary step in understanding the effect of the active ingredients present in the leaves of this plant, we have undertaken a phytochemical analysis of ethanolic extract and its successive fractions with the solvents, petroleum ether, chloroform, ethyl acetate, n-butanol and methanol.

MATERIALS AND METHODS:

The leaves of *H. integrifolia* were obtained from a local forest of Mangaluru. The plant was authenticated by Dr. K. V. Nagalakshamma, Head, Dept. of Botany, St. Aloysius College, Mangaluru. A voucher specimen was deposited in the herbarium of NGSM Institute of Pharmaceutical Sciences, Paneer, Deralakatte, Mangaluru.

The leaves were shade dried at room temperature and pulverized to course powder. The powdered plant material was extracted with 95% v/v ethanol by cold-maceration method for four days. The method was repeated for four times. The extract was filtered through muslin cloth and concentrated to one third of its initial volume by using rotary evaporator (Superfit, India) at 40°C. The remaining solvent was evaporated completely using water bath under 40°C to dryness. The dried ethanolic extract of plant material was then subjected for successive fractionation with solvents.

The extract (400g) was suspended in distilled water (1,500 ml) and then extracted successively with petroleum ether (60 – 80°C, 8500ml), chloroform (8500ml), ethyl acetate (8500ml), n-butanol (8500ml) and methanol (8500ml). All the fractions were then washed with distilled water (30 ml), dried over anhydrous sodium sulphate and freed of solvent by distillation. The ethanolic extract was thus fractionated into petroleum ether soluble extract (40g), chloroform soluble extract (66g), ethyl acetate soluble extract (78g) n-butanol soluble extract (87g) and methanol soluble extract (114g).

Preliminary phytochemical studies were performed for all the extracts according to the standard procedures (7).

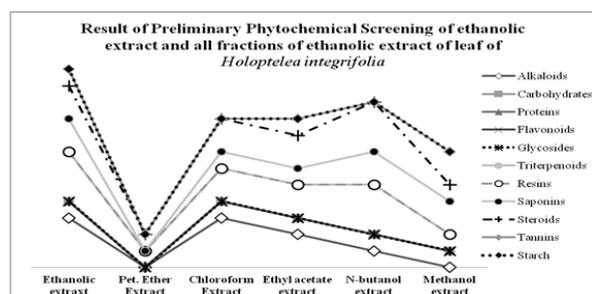
RESULTS:

TABLE-1. Result of preliminary phytochemical analysis of ethanolic, petroleum ether, chloroform, ethyl acetate, n-butanol and methanolic extracts of *H. integrifolia* leaf.

Constituent	Test	Inference					
		Ethanolic extract	Pet. Ether Extract	Chloroform Extract	Ethyl acetate extract	N-butanol extract	Methanol extract
Alkaloids	a) Dragendorff's test	+++ve	-ve	+++ve	++ve	+ve	-ve
	b) Hager's test	+++ve	-ve	+++ve	++ve	+ve	-ve
	c) Wagner's test	+++ve	-ve	+++ve	++ve	+ve	-ve
	d) Mayer's test	+++ve	-ve	+++ve	++ve	+ve	-ve
Carbohydrates	a) Anthrone test	+ve	-ve	+ve	+ve	+ve	+ve
	b) Benedict's test	+ve	-ve	+ve	+ve	+ve	+ve
	c) Fehling's test	+ve	-ve	+ve	+ve	+ve	+ve

	d)	Molisch test	+ve	-ve	+ve	+ve	+ve	+ve
Proteins	a)	Biuret test	-ve	-ve	-ve	-ve	-ve	-ve
	b)	Millon's test	-ve	-ve	-ve	-ve	-ve	-ve
	a)	Shinoda's test	-ve	-ve	-ve	-ve	-ve	-ve
Flavonoids	a)	Molisch test	-ve	-ve	-ve	-ve	-ve	-ve
	b)	Borntrager's test	-ve	-ve	-ve	-ve	-ve	-ve
	c)	Modified Borntrager's test	-ve	-ve	-ve	-ve	-ve	-ve
Triterpenoids	a)	Liebermann-Burchard's test	+++ve	+ve	+++ve	+++ve	+++ve	+ve
Resins			-ve	-ve	-ve	-ve	-ve	
Saponins			+++ve	+ve	+ve	+++ve	+++ve	
Steroids	a)	Liebermann-Burchard's test	+++ve	+ve	+++ve	+++ve	+++ve	+ve
	b)	Salvoski test	+++ve	+++ve	+ve	-ve	+ve	-ve
Tannins			+ve	-ve	+ve	-ve	+++ve	
Starch			-ve	-ve	-ve	-ve	-ve	

FIG. 1. Graphical representation of the result of preliminary phytochemical analysis of ethanolic, petroleum ether, chloroform, ethyl acetate, n-butanol and methanolic extracts of *H.integrifolia* leaf.



The detailed results of preliminary phytochemical analysis of different extracts of *H.integrifolia* leaf are explained in Table-1. Presence of alkaloids, carbohydrates, triterpenoids, saponins, steroids and tannins were demonstrated in the ethanolic extract. Ethyl alcohol extract also showed the presence of all these ingredients. Chloroform and n-butanol extracts showed alkaloids, triterpenoids, saponins and steroids. Methanolic extract demonstrated the presence of triterpenoids, saponins, steroids and tannins whereas petroleum ether extract showed the presence of triterpenoids and steroids.

Fig-1 shows a graphical representation of the same results. It may be observed that the ethanolic extract contained the phytochemical ingredients in higher quantity compared to the other extracts. Alkaloids are highest in ethanolic extract followed by chloroform, ethyl acetate and n-butanol extracts. Terpenoids, saponins, steroids, tannins were also seen in higher levels in ethanolic extract. Among the other extracts, chloroform, ethyl acetate and n-butanol fractions showed better results followed by methanolic extract.

DISCUSSION:

Tennis elbow usually presents as a small area of chronic pain and swelling on the lateral aspect of the elbow due to repeated contraction of fore arm muscles which may lead to tiny tears in the tendons that attach the fore arm muscles to the bony prominence at the outside of the elbow (8). Painkillers and non-steroidal anti-inflammatory drugs are commonly used in treating tennis elbow. Alkaloids, flavonoids and terpenoids present in various parts of the plants show anti-inflammatory activities (9). Anti-inflammatory effect is mediated through the regulation of various inflammatory cytokines, such as nitric oxide, interleukins, tumor necrosis factor alpha- α , interferon gamma- γ as well as noncytokine mediator, prostaglandin E₂(10). Analgesic drugs usually act by inhibiting the synthesis of prostaglandins (11). In a review, Ruhil P *et al*, described that methanolic and petroleum ether extract of leaves and bark of *H.integrifolia* showed antibacterial, antioxidant, antidiabetic and antitumor activities with identification of phytochemicals, alkaloids, steroids, tannins, phenol and proteins (12). Maximum analgesic activity was observed by the crude ethanolic extract of *H.integrifolia* in a comparative study on experimental animals (6). We have done extraction of active ingredients in the leaf of *H.integrifolia* using various solvents and tried to do a gross comparison of the efficiency of different solvents in extracting the phytochemical ingredients.

In the present study, we have identified the presence of alkaloids, carbohydrates, triterpenoids, saponins, steroids and tannins in the

ethanolic leaf extract of *H.integrifolia*. Ethyl acetate extract also demonstrated similar phytochemical components but the concentration was lesser compared to ethyl alcohol. Triterpenoids and steroids were effectively extracted using n-butanol whereas alkaloids, triterpenoids and steroids were effectively extracted by chloroform. Thus it may be opined that ethanol and ethyl acetate are better solvents in extracting the phytochemical ingredients compared to others. Alkaloids, triterpenoids, saponins, steroids and tannins present in *H.integrifolia* leaves may be responsible for the proposed activity on curing tennis-elbow. However, a detailed controlled study about the same effect and about the phytochemical responsible for the activity is envisaged.

CONCLUSION:

Ethanol is observed to be a good solvent in extracting the active principles from the leaves of *H.integrifolia* where the extract demonstrated the presence of alkaloids, triterpenoids, saponins, steroids and tannins followed by chloroform, ethyl acetate and n-butanol. Petroleum ether and methanol appeared to be less efficient as solvents in the extraction of the same. The anti-inflammatory and analgesic activity of these phytochemical ingredients may be responsible for the predictive role of *H.integrifolia* in curing Tennis elbow.

ACKNOWLEDGEMENT:

We thank the Principal, Prof. (Dr.) C. S. Shastry and the Department of Pharmacognosy of NGS Institute of Pharmaceutical Sciences, Paneer, Deralakatte, Mangaluru for supporting the study.

CONFLICTS OF INTEREST:

None.

REFERENCES

- Kumar D., Kumar K., Gupta J., Bishnoi N., and Kumar S. (2012). "A mini review on chemistry and biology of *Holoptelea integrifolia* Roxb. Planch (Ulmaceae)". *Asian Pacific Journal of Tropical Biomedicine*, 5(12):200-5.
- Sharma S., Khatri P., Pandey A., Jakhethia V., Chaturvedi C., and Dwivedi N. (2010). "Antidiabetic screening of leaves extract of *Holoptelea integrifolia*". *International Journal of Pharmaceutical Research and Development*, 2:66-71.
- Kalpana, and Upadhyay A. (2010). "Anti-inflammatory evaluation of ethanolic extract of leaves of *Holoptelea integrifolia* Planch". *Annals of Biological Research*, 1(2):185-195.
- Lakshmi K.S., Sharma S.S., Rajesh T., and Chitra V. (2010). "Antitumor activity of ethanolic extract of leaves of *Holoptelea integrifolia* on Dalton's ascetic lymphoma in Swiss albino mice". *International journal of green pharmacy*, 4:44-47.
- Sharma S., Lakshmi K.S., Patidar A., Chaudhary A., and Dhaker S. (2009). "Studies on anti-inflammatory effect of aqueous extract of leaves of *Holoptelea integrifolia*, Planch in rats". *Indian Journal of Pharmacology*, 41(2):87-88.
- Rizwani G.H., Mahmud S., Shareef H., Perveen R., and Ahmed M. (2012). "Analgesic activity of various extracts of *Holoptelea integrifolia* (Roxb) planch leaves". *Pakistan Journal of Pharmaceutical Sciences*, 25(3):629-632.
- Harbone J.B. (1998). "Phytochemical methods: a guide to modern techniques of plant analysis", 3rd edition Thompson Publishing IT, 5-21.
- Flat A.E. (2008). "Tennis elbow". *Proceedings (Baylor University. Medical Center)*, 21(4):400-402.
- Lewis D.A. (1995). "In:Anti-Inflammatory Drugs from Plant and Marine Sources". Birkhauser-Verlag, 95-227.
- Zhu F., Du B., and Xu B. (2018). "Anti-inflammatory effects of phytochemicals from fruits, vegetables, and food legumes: A review". *Critical Review on Food Science and Nutrition*, 58(8):1260-1270.
- Eldahshan O.A., and Abdel-Daim M.M. (2015). "Phytochemical study, cytotoxic, analgesic, antipyretic and anti-inflammatory activities of *Strychnos nux-vomica*". *Cytotechnology*, (67):5:831-844.
- Ruhil P., and Minocha N. (2017). "Recent advances in pharmacological potential & phytochemical screening of *Holoptelea integrifolia*: a review". *International Journal of Scientific Research*, 6(12):458-459.