http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 4 (2): 166-168, 2001

Chemical and Biological Evaluation of Essential Oil of *Teclea nobilis* Leaf

Adnan J. Al-Rehaily
Department of Pharmacognosy and Medicinal, Aromatic and Poisonous Plants Research Center, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh-11451, Saudi Arabia

Abstract: The chemical composition of the essential oil of the leaves of *Teclea nobilis* Defile (Rutaceae) (TN), was investigated by using GC/MS. Out of fifty four peaks (representing 99.3% of the oil), twenty three components were identified, representing 62.3% of the total oil composition. The main components were Germacrene-d (19%), Ocimene isomer (13%), Guaiol (3.9%), Elemol (2.9%) and Bulnesol (2.5%). Analgesia was evident in both the acetic acid induced writhing and tail flick reaction time tests in mice. A significant antipyretic activity of oil was also observed in mice. A slow and gradual neuromuscular blocking effect was recorded on rat's phrenic-nerve diaphragm preparation. The oil showed no anti-microbial potential on various microorganisms tested. The oil showed sedative effect on behavioural tests without causing any side effect.

Key words: *Teclea nobilis*, essential phytochemistry, pharmacology

Introduction

The leaves and bark of *Teclea nobilis* Defile (Rutaceae) locally called Al-Thureim are known in ethnomedicine having properties to reduce pain and fever (Hedberg *et al*., 1989). The presence of alkaloids has been described in this plant (Yenesew and Dagne, 1988). In an earlier study of Mascolo *et al*. (1988), analgesic and antipyretic activities of ethanolic extracts have been reported. As no phytochernical and pharmacological studies on the essential oil of *T. nobilis* are reported, it was thought worthwhile to explore out its chemical and biological effects.

Materials and Methods

Plant Material: leaves of *Teclea nobilis* were collected from Tannumah (An Nimes). southern province, Saudi Arabia, in March 2000. The plant was identified by Dr. Sultanul Abidin and a voucher specimen is kept at the Herbarium, College of Pharmacy, King Saud University.

Analysis of the essential oil: The fresh leaves were used for the preparation of essential oil by Hydrodistillation. Analysis of the oil was performed by GC/MS under the following conditions: A Hewlett-Packard 5973 MSD GC/MS equipped with 30 m×0.32 mm XTI-5 microfilm capillary column; oven temp. 40°C (hold 3 min) to 200°C at 8°C/min. then to 320°C at 15°C/min (hold 4 min); injector temp. 320°C; sample size 0.05 μl; split 1:100; mass range 35-500 amu, 2.78 scans/sec. Oil components were identified by comparing their retention times and mass spectral data with authentic samples.

Biological tests: Antipyretic activity - An increase in body temperature was induced in mice by subcutaneous injection of 20 ml/kg of a 20% aqueous suspension of brewer's yeast according to (Loux *et al.,* 1972). The mean rectal temperature was measured at different intervals after i.p. administration of the oil or indomethacin.

Analgesic activity: inhibition of acetic acid-induced writhing in mice: The test was carried out using the technique (Siegmund *et al*., 1957) as modified by (Koster *et al.,* 1959). The oil was administered orally, to 16 h fasted mice, divided into groups of six animals each. One hour after treatment, the mice were injected intraperitoneally with 0.2 ml of 3% acetic acid solution to induce the characteristic writhings. The number of writhings occurring between 5 and 15 min after the acetic acid injection was recorded. The responses of oil-treated groups were compared with those of animals receiving indomethacin (as standard drug), as well as with the controls.

Tall flick Test: Acute nociception was assessed using a tail flick apparatus (Tail Flick model DS 20 Sorrel Apelex, France) following the method D'Amour and Smith. Briefly, each animal was placed in a restrainer, 2 min before treatment and baseline reaction time was measured by focusing on intensity controlled beam of light on the distal one-third portion of the animals tail. The oil was orally administered immediately after this step and 25 min later, the post drug reaction time was measured. A 10 seconds cut off time was used in order to prevent tissue damage.

Studies on rat's diaphragm phrenic nerve preparation: The phrenic nerve was stimulated at a rate of 12 shocks per minute by rectangular wave pulses of about 0.5 msec duration and the contractions of the diaphragm were recorded on a physiograph. The oil was added in the bath and washed for recovery (Bulbring, 1946).

Evaluation of Antimicrobial Activity: The rapid antimicrobial screening program used is essentially the agar dilution method described by Mitscher *et al.* (1972).

Behavioural Studies: Behavioural studies were carried out in mice, weighing 25-30 g, according to the scheme of Irwin (1964). The oil was administered intraperitoneally and the animals were observed for excitation, tremors, twitches, motor activity, pinna, corneal reflexes, and respiratory changes.

Acute Toxicity Studies: The oil was administered by oral route to various (one group served as control) groups of 10 mice (5 male and 5 female), after an overnight fast. The doses studies were 0.05. 0.1, 0.5, and 1.0 ml/kg body weight. The animals were observed for seven consecutive days to register

Table 1: Identified constituents in the essential oil of *Teclea nobilis* Defile

Peak No.	Retention time minutes	Compound	% Area
1.	10.53	beta-Myrcene	0.1
2.	11.54	Ocimene isomer	8.8
3.	11.77	Ocimene isomer	13
4.	12.64	Linatool	1.6
5.	13.43	Ocimene isomer	0.5
6.	13.68	Epoxyocimene	0.2
7.	16.61	Dihydroedulan II	1.0
8.	16.71	Dihydroedulan 1	0.5
9.	18.12	alpha-Copaerte	0.6
10.	18.30	beta-Bourbonene	0.4
11.	18.34	Elemene	1.5
12.	18.61	Cyperene	0.2
13.	18.64	Methyl-N-methyl	0.3
14.	18.90	beta-Caryophyllene	0.9
15.	19.46	alpha-Humulene	1.3
16.	19.90	Germacrene-d	19
17.	20.45	delta-Cadinene	1.9
18.	20.78	alpha-Copaene- 11-ol	0.7
19.	20.86	Elemol	2.9
20.	21.40	Spathulenol	0.2
21.	21.63	Guaiol	3.9
22.	22.67	Bulnesol	2.5
23.	23.93	Benzyl benzoate	0.3

Table 2: Effect of essential oil of *Teclea nobilis* on east induced hyperprexia in mice

Group (n =6)	Dose mg/kg	Pre drug	Rectal Temperature				
			15 min	30 min	60 min	90 min	120 min
Teclea oil	10 µl	38.2±0.36	37.85±0.32	37.43±0.23	37.01±0.10**	37.03±0.16*	37.2±0.15*
Indomethacin	4	38.2±0.28	37.00±0.30*	36.2±0.28***	35.43±0.30***	35.6±0.20***	36.03±0.08***

Table 3: Effect of essential oil of *Teclea nobilis* on acetic acid induced writhin

Group (n =6)	Dose mg/kg	Number of withings Mean±S.E.	% Inhibition
Control Acetic Acid	0.2 ml of 3%	39.5±1.25	
Teclee nobilis oil	10 µl	32.33±1.82*	18%
Indomethacin	4	16.66±1.85***	58%

*p<0.05; ***p<0.001 Student's t-test.

Table 4: Effect of essential oil of *Teclea nobilis* on tail flick test in mice

Group (n =6)	Dose mg/kg	Pre drug	Rectal Temperature				
			15 min	30 min	60 min	90 min	120 min
Tooled, nobilis oil	10 µl	2.88+0.06	3.71±0.08***	4.13±0.09***	4.08±0.07***	3.98±0.15***	3.91±0.09***
Indomethacin	4	2.9±0.05	3.88±0.09***	4.41±0.07***	4.38 ±0.09***	4.46±0.16***	4.18±0.14***

*p<0.001; Student's t-test.

mortality or other toxic symptoms (Al-Yahya *et al*., 1994)

Results

The fresh leaves of *Teclea nobilis* yielded 0.23% v/w of essential oil. Table 1, summarized the constituents identified by GC/MS analysis, their retention times and area percentage are also tabulated. Sesquiterpene and monoterpene hydrocarbons were the major constituents in the oil. They constituted 25.2 and 22.6%, of the total oil content respectively. Germacrene-d (19%) is the major sesquiterpene hydrocarbon, while Ocimene isomers (22.3%) are the major monoterpene hydrocarbons.

On the other hand, oxygenated sesquiterpenes namely, Guaiol (3.9%), Elemol (2.9%) and Bulnesol (2.5%) were detected in appreciable amount.

In addition, minor components were detected of which Linalool (1.6%), Dihydredulenol II (1.0%), Methyl-N-methylanthranilate (0.3%) and Benzyl benzoate 10.3%) were identified.

The elevated rectal temperature of mice followed by subcutaneous injection of brewer's yeast was found to be decreased by intraperitoneal administration of essential oil of TN (Table 2). The decrease in rectal temperature was found to

Fig. 1: Effect of essential oil of Teclea nobilis on responses of the rat diaphragm to stimulation of the phrenic nerve

be statistically significant. There is a highly significant analgesic activity was recorded in both the models (writhing and tail flick) used (Table 3, 4) in groups of mice treated with TN oil. On rat's diaphragm phrenic nerve preparation, the oil showed a slow and gradual neuromuscular blocking activity (Fig. 1).

The microbiological studies showed no anti-microbial potential on micro-organisms used.

The observation of animals after the intraperitoneal administration of oil, has shown a CNS depressant activity. A single nign close of TN oil administration did not cause any deleterious effect or mortality of the animals except sedation for a longer period.

Discussion

The data presented in this study demonstrated several points. The plant is producing good amount of oil represented by 4 ml in 1.750 kg leaf extract. This means that the plant is relatively rion in oil and fat constituents. In fact this was observed earlier with alcohol extract of the plant (Unpublished fesults) that the dried extract was loosing measurable amount (about 5 g) after one day left in the desicator. The oil of *Teclea nobilis* is characterized by the presence of high percentages of sesquiterpene hydrocarbons than other essential oil constituents. In addition, the bulk of the identified alcohols are of sesquiterpene nucleus. Also no work has been done on the essential oil of any of *Teclea* species. With all these findings, it can be concluded that these informations may provide a point of interest in the chemotaxonomy of this species. The present study assessed the biological effects of essential oil of *T. nobitis* a medicinal plant reputed in African and Arabian traditional medicine for its analgesic. antipyretic properties (Watt and Breyer-Brandwijk, 1962). The oil has exerted pharmacological effects including analgesic activity in mice. Pretreatment of mice with oil of TN reduced writhing induced by acetic acid indicating the presence of active substances in oil endowed with analgesic activity. This action of oil could be due to its CNS depressant activity as observed in the behavioural studies. Essential oils are known to produce CNS depressant and sedative effects both in animals and humans (Lis-Balchin and Hart, 1994). On the other hand. an antipyretic activity again confirms its febrifugal property (Mascolo *et al.,* 1988). The hypothermic effect in rectal temperature of animals may be due to peripheral vasodilation (Mishra and Agrawal, 1988). The effect of TN essential oil on skeletal muscle has reduced the twich response to phrenic nerve stimulation and exerted a slow and gradual neuromuscular blocking. This action may be attributed to the components present in the oil acted by a post junctional block of neuromuscular transmission (Bowman, 1990). It has also been reported that many essential oils have been claimed to possess muscle relaxant action (Lis-Balchin and Hart, 1997). The TN oil was found to be safe in acute toxicity test and showed no deleterious effects in mice except sedation. in conclusion, the results of the present study support the ciaims of traditional medicine practitioners, the use of TN for relieving pain and in feverish conditions.

References

Al-Yahya, M.A., J.S. Mossa, A.M. Ageel and S. Rafatullah, 1994. Pharmacological and safety evaluation studies on *Lepidium sativum* L., seeds. Phytomedicine, 1: 155-159.

Bowman, W.C., 1990. Pharmacology of Neuromusculae Transmission. Butterworth-Heinemann, London, pp: 239-242.

Bulbring, E., 1946. Observations on the isolated phrenic nerve diaphragm preparation of the rat. Br. J. Pharmacol. Chemother., 1: 38-61.

Hedberg, I., S. Edwards and S. Nemomissa, 1989. Flora of Ethiopia. Vol. 3, Uppsala University, Sweden.

Irwin, S., 1964. Pharmacologic Techniques in Drug Eveluation. Year Book Publication, USA.

Koster, R., M. Anderson and E.J. de Beer, 1959. Acetic acid-induced analgesic screening. Fed. Proc., 18: 412-412.

Lis-Balchin, M. and S. Hart, 1997. A preliminary study of the effect of essential oils on skeletal and smooth muscle *in vitro*. J. Ethnopharmacol., 50: 185-187.

Lis-Balchin, M.T. and S.L. Hart, 1994. A pharmacological appraisal of the folk medicinal usage of *Pelargonium grossularioides* and *Erodium cicutarium*. J. Herbs Spices Med. Plants, 2: 41-48.

Loux, J.J., P.D. de Palma and S.L. Yankell, 1972. Antipyretic testing of aspirin in rats. Toxicol. Applied Pharmacol., 22: 672-675.

Mascolo, N., A. Pinto, F. Capasso, A. Yenesew and D. Ermias, 1988. Antipyretic and analgesic studies of the ethanalic extract of *Teclea nobilis* delile. Phytother. Res., 2: 154-156.

Mishra, P. and R.K. Agrawal, 1988. Some pharmacological actions of the essential oil of *Luvanga* scandens. Fitoterapia, 59: 441-448.

Mitscher, L.A., R.P. Leu, M.S. Bathala, W.N. Wu and J.L. Beal, 1972. Antimicrobial agents from higher plants. I. Introduction, rationale and methodology. Lloydia, 35: 157-166.

Siegmund, E., R. Cadmus and G. Lu, 1957. A method for evaluating both non-narcotic and narcotic analgesics. Proc. Soc. Exp. Biol. Med., 95: 729-731.

Watt, J.M. and M.G. Breyer-Brandwijk, 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa. 2nd Edn., E and S Liningstone Ltd., London, UK., Pages: 1457.

Yenesew, A. and E. Dagne, 1988. Alkaloids of *Teclea nobilis*. Phytochemistry, 27: 651-653.