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Extraction and Isolation of Chemical Constituents from *Schima Wallichii* Bark

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Abstract

The methanol extract of bark of *Schima wallichii*, vernacularly known as 'Chilaune' was subjected to column chromatography. From GC-MS, 2, 3-benzofurandione, Phenylpropanolamine, Glycidol and Rotenone were found to be present in a fraction obtained from column chromatography.

Keywords: *Schima wallichii*, Column Chromatography, GC-MS, 2, 3-benzofurandione, Phenylpropanolamine, Glycidol and Rotenone.

Introduction

Nepal is rich in all three levels of biodiversity namely species diversity, genetic diversity and habitat diversity. It has many plants with medicinal and aromatic values. Among the 7000 species of medicinal plants recognized all over the world, more than 900 types of precious medicinal plants are found in Nepal[1]. The plant *Schima wallichii (Chilaune) is one of the most important species of genus Schima which belongs to the family Theaceae. Schima* is an evergreen tree up to 35 m tall. *The genus name is derived from the Greek word 'skiasma' (shadow), probably referring to the dense crown. Schima grows in moist and dry evergreen as well as in mixed deciduous forests[2]. Once considered to contain up to fifteen species like* S. superba, S. noronhae etc., *it is now placed them all under Schima wallichii (DC.) Korth by S. Bloembergen in 1952[3]. Schima has a fast growth even under infertile soil conditions. Flowering is in April-May and fruiting is in February-March. The genus inhabits warm temperate to subtropical climates across southern and southeastern Asia*[2, 3]. The bark contains an alkaloid used as a fish poison. The young plants, leaves and roots are used medicinally against fevers. The astringent corollas are used to treat uterine disorders and hysteria. In Nepal, the leaves are also used for fodder[4, 5]. The bark of this plant is traditionally used as antipyretic, antiseptic, anthelmintic and wound healing agent[6]. Previously research reported that *Schima wallichii* leaves have a cytotoxic activity and it is a medicinal plant which is potential to be developed as anticancer[7,8]. The present study deals with the chemical analysis of methanol extract of bark of *Schima wallichii* of Gulmi district of Nepal.

Experimental methods

General

Melting points were determined by using melting point apparatus from Griffin and George Company Limited (UK). Column chromatography was performed using silica gel of mesh no. 60-120. For thin layer chromatography "Silica gel-G" was used. EI-MS measurements were carried out on BioTOF II triple quadruple mass spectrophotometer.

Plant materials

The bark of *Schima wallichii* was collected from Gulmi district of Nepal in February, 2012. The plant was identified by comparison with authentic herbarium from Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

Extraction

100 gm of bark of *Schima wallichii* was completely extracted by percolation methods using methanol as a solvent at room temperature to obtain methanol extract. The solvent from plant extract was removed by evaporation with the help of Rotary evaporator at high pressure maintaining temperature lower than the boiling point of the respective solvent used and left for drying in 100 mL beaker.

Separation of compounds by the use of column chromatography:

The methanol fraction (14 gm) was adsorbed on 17 gm of silica gel and loaded on to a silica gel (112 gm, E-merck, 60-120 mesh) packed in the column having internal diameter 3 cm with the adsorbent height 33 cm. The column was eluted with gradients

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of chloroform ($CHCl_3$) and methanol (MeOH) to obtain a number of fractions.

Table: Elution and TLC of different fractions of methanol extract

S.N.	Eluents	Fraction No.	Volume of Eluents (mL)	Solvent system for TLC	TLC Report
1	100 % Chloroform	1-20	2000	10 % EtOAc in $CHCl_3$	Tailing
2	5 % MeOH in $CHCl_3$	21-34	1400	50 % EtOAc in $CHCl_3$	Tailing
3	10 % MeOH in $CHCl_3$	35-40	600	50 % EtOAc in $CHCl_3$	Tailing
4	20 % MeOH in $CHCl_3$	41-45	500	50 % EtOAc in $CHCl_3$	Tailing
5	20 % MeOH in $CHCl_3$	46-50	500	10 % MeOH in $CHCl_3$	Spot + Tailing
6	20 % MeOH in $CHCl_3$	51-57	800	20 % MeOH in $CHCl_3$	Spot + tailing
7	20 % MeOH in $CHCl_3$	58-64	700	20 % MeOH in $CHCl_3$	tailing
8	20 % MeOH in $CHCl_3$	65-69	500	30 % MeOH in $CHCl_3$	Tailing
9	20 % MeOH in $CHCl_3$	70-79	1000	30 % MeOH in $CHCl_3$	Tailing
10	30 % MeOH in $CHCl_3$	80-84	500	30 % MeOH in $CHCl_3$	Tailing + spots
11	30 % MeOH in $CHCl_3$	**85-92**	700	35 % MeOH in $CHCl_3$	4 spots(m)
12	30 % MeOH in $CHCl_3$	93-105	1300	35 % MeOH in $CHCl_3$	No spot
13	40 % MeOH in $CHCl_3$	106-137	3200	35 % MeOH in $CHCl_3$	Tailing
14	40 % MeOH in $CHCl_3$	138-158	2100	40 % MeOH in $CHCl_3$	Tailing
15	40 % MeOH in $CHCl_3$	159-170	1200	40 % MeOH in $CHCl_3$	No spot
16	50 % MeOH in $CHCl_3$	171-183	1300	50 % MeOH in $CHCl_3$	Tailing
17	60 % MeOH in $CHCl_3$	184-188	500	80 % MeOH in $CHCl_3$	Tailing

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18	80 % MeOH in $CHCl_3$	189-193	500	80 % MeOH in $CHCl_3$	Tailing
19	100 % MeOH	194-197	400	80 % MeOH in $CHCl_3$	Tailing

Isolation and Characterization of Compounds:
Fractions 85-92 (SW), eluted with 30 % MeOH in $CHCl_3$ were found identical in TLC with four major spots therefore, they were mixed and concentrated under reduced pressure. The concentrated mass (107 mg) was slightly soluble in methanol and insoluble in less polar solvents like hexane, ethyl acetate (EtOAc) and chloroform. Then it was subjected to GC-MS for identification of the compounds. GC-MS was conducted in Department of Plant Resources, Thapathali, Kathmandu.

Isolation and identification of compounds from *Schima wallichii* bark:

Mixture of fractions 85-92 (SW), obtained after column chromatography of the methanol extract of the bark of *Schima wallichii*, eluted by the solvent 30 % methanol in chloroform, was subjected first to pre-coated TLC and then to GC-MS. Four major spots were observed under TLC. From GC-MS following four compounds were identified;

- 2, 3-benzofurandione
- Phenylpropanolamine
- Glycidol
- Rotenone

Results and discussion

Compounds, Molecular weight, Retention time and Area (%) are given as;

S.N.	Compounds	Mol. Wt.	R. Time (min)	Area (%)
1	2, 3-benzofurandione	148	13.908	22.120
3	Phenylpropanolamine	151	18.131	18.440
2	Glycidol	74	16.198	17.140
4	Rotenone	394	21.165	7.110

Major compounds present in 85-92 fractions were 2, 3-benzofurandione (22.120 %), Phenylpropanolamine (18.440 %) and Glycidol (17.140 %).

Analysis of Mass Spectrum of Major Compound 1 (2, 3-benzofurandione):
The mass fragmentation pattern of the compound **1** (2, 3-benzofurandione) can be described as below [9]. The base peak of the spectrum is at m/z = 120. This ion subsequently eliminate carbon monoxide to give a strong peak at m/z = 92 and then at m/z = 65.

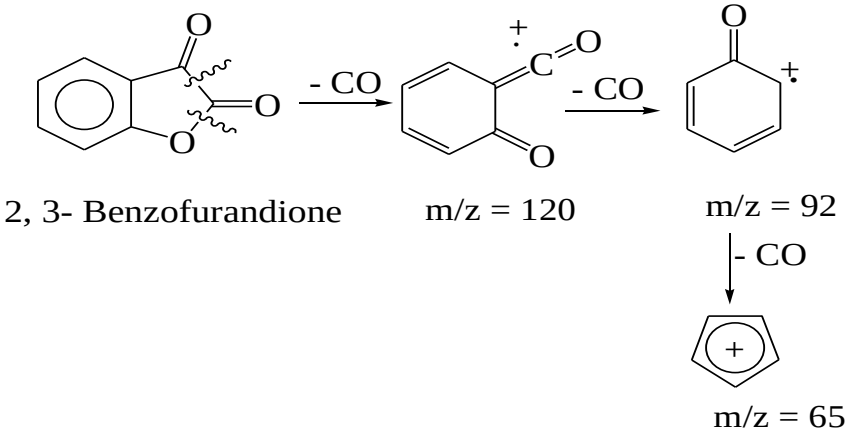

Fig.1. Mass fragmentation pattern of 2, 3-benzofurandione

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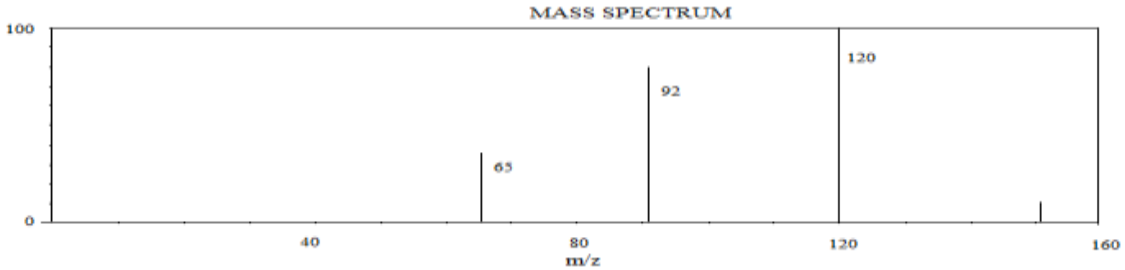

Fig.2. Mass spectrum of 2, 3-benzofurandione

Analysis of Mass Spectrum of Major Compound 2 (Phenylpropanolamine):

The mass spectrum of Compound **2** (Phenylpropanolamine) showed the base peak at m/z = 44 representing $[CH_3\text{-}CH{=}NH_2]^+$ fragment along with less intense peaks at m/z = 107. Similarly other prominent peaks were obtained at m/z = 107, 79 and 77. The fragmentation pattern can be represented as: [6]

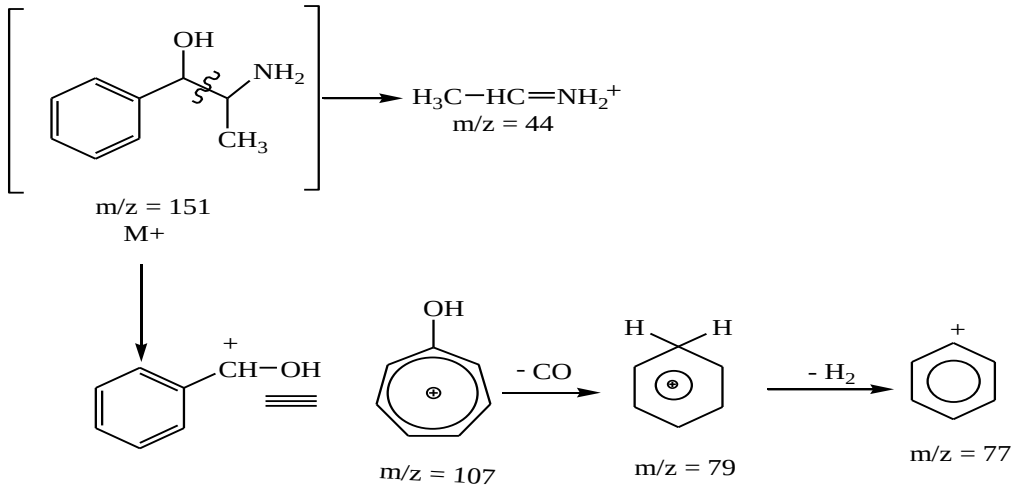

Fig.3. Mass fragmentation pattern of Phenylpropanolamine

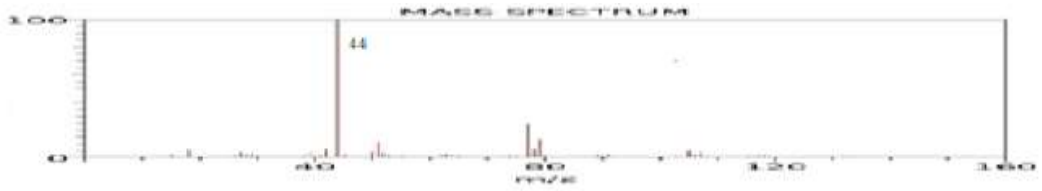

Fig.4. Mass Spectrum of Phenylpropanolamine

Analysis of Mass Spectrum of Major Compound 3 (Glycidol):

The mass spectrum of Compound **3** (Glycidol) showed base peak at m/z = 57 along with peaks at m/z = 43 and m/z = 44.

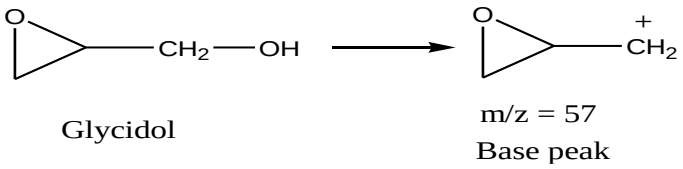

Fig.5. Fragmentation pattern in Glycidol (for base peak)

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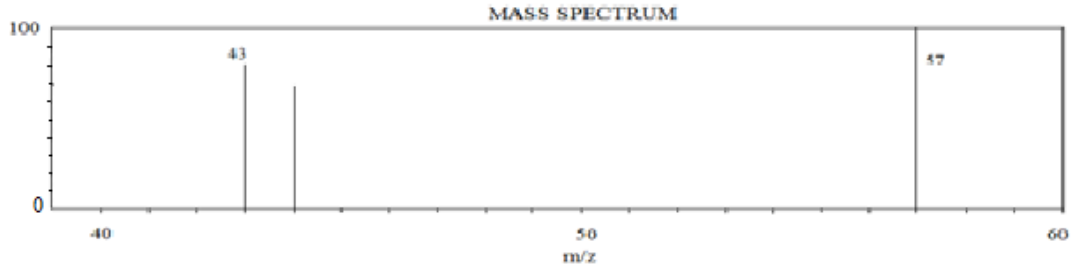

Fig.6. Mass Spectrum of Glycidol

Analysis of Mass Spectrum of Compound 4 (Rotenone):
The Mass spectrum of Compound **4** (Rotenone) obtained from GC-MS is given as;

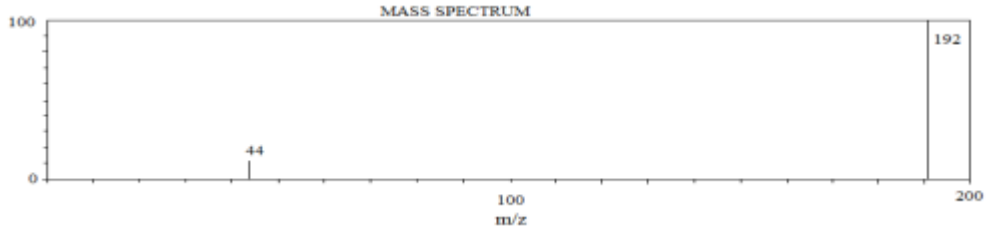

Fig.7. Mass Spectrum of Rotenone

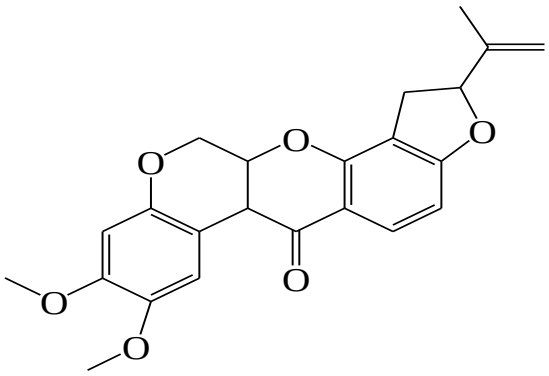

Fig.8. Molecular structure of Rotenone

Conclusion

From GC-MS, compounds like 2, 3-benzofurandione, Glycidol, Phenylpropanolamine and Rotenone were suggested to be present in fractions 85-92 of 30 % MeOH in chloroform eluted from column chromatography of methanol extract of *Schima wallichii*.

Acknowledgements

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References

1. Manandhar NP, Plants and People of Nepal. Timber Press, USA. 2000 ; 50.
2. Wikipedia, the Free Encyclopedia. (2013, March 19). Schima. [Online]. (URL http://en.wikipedia.org/wiki/Schima). (Accessed 9 February, 2013).
3. McNamara W. A., Quarryhill Botanical Garden, (1966), *Schima*. [Online]. (URL http://www.quarryhillbg.org/page15.htmL). (Accessed 9 March, 2013).
4. World Agroforestry Centre, an Agroforestrytree Database (2004), *Schima wallichii*. [Online]. (URL

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http://www.worldagroforestrycentre.org/sea/products /afdbases/af/asp/SpeciesInfo.asp?SpID=1491). (Accessed 3 March 2013).

5. Jackson J K, (1994). *Manual of Afforestation in Nepal*. [Online]. (URL http:// www.forestrynepal.org/resources/trees/schima-wallichii?quicktabs_11=6) (Accessed 3 March 2013).
6. Das S, Ghosh LK, Evalutaion of analgesi, antipyretic and anti inflammatory activity of different fractions of *Schima wallichii* bark, Pharmacologia. 2013; 4**:**400- 403.
7. Kushimizu, K. Murakami, A. Hayashi, H. Ohigashi, H. Subarnas, A. Gurmaya, K.J. and Ali, A.M. (1998) . Biological activity of edible and medicinal plants from Indonesia and Malaysia: In: The Tokyo International Forum on Conservation and Sustainable use of Tropical Bioresources, Japan Bioindustry Association, Tokyo,203-208.
8. Subarnas A, Hadianyah C, Gurmaya KJ, Muhtadi A, Characteriaztion of antimutagenic compound from primates-consumed plants *Schima wallichii*, *Biotika* 2003; 2**:**7-13.
9. Silverstein RM, Webster FX, "Spectrometric Identification of Organic Compounds", sixth edition, Gopal Jee Enterprises, Delhi, India, 2007.