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Short Communication

Isolation of Aromatic Sulphone from *Plumbago zeylanica* Linn.

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Abstract. Diphenyl sulphone and β-sitosterol were isolated from the petroleum ether extract of stem of *Plumbago zeylanica* Linn. They were characterized by spectroscopic methods. Isolation of diphenyl sulphone from this plant is the first report from higher plants. Diphenyl sulphone was tested for cytotoxicity by brine shrimp lethality test.

Keywords: *Plumbago zeylanica*, plumbaginaceae, diphenyl sulphone

Plumbago zeylanica is a member of family Plumbaginaceae which is commonly known as "white flower leadwort" (English) and "Chitu" in Nepali. It is distributed in Nepal from 100-1300 m altitude and as a medicinal plant is used for curing cancer and malignant tumors in India, Indonesia, Philippines, Nigeria (native medicine), Pakistan, Ghana and France (Hartwell, 1970). Literature survey has revealed that a number of phytochemical groups like napthoquinones, sterols, triterpenoids (Gupta *et al.*, 1995, Qian and Zhou, 1980) and aromatic acids (Qian and Zhou, 1980) were isolated from its aerial parts. Keeping in view the biological/pharmacological importance of this plant, present studies were undertaken on the phytochemical analysis of its stem.

The plant was purchased in April, 2000 from the local market. Its identity was authenticated and a voucher specimen was deposited at the Central Herbarium of Central Department of Botany, Tribhuvan University, Nepal, under code number TUCH - 11.

The air-dried stems (500 g) were chopped, finely powdered and extracted with petroleum ether (60-80 °C), (4 l x 16 h) in a Soxhlet extractor. The extract was filtered and evaporated giving a greenish yellow residue (1.81 g). The residue (1.75 g) was chromatographed on silica gel column (2.3 x 40 cm, 40 g) by eluting with hexane-chloroform gradient. Fractions eluted with hexane-chloroform (85:15-70:30) were concentrated and left overnight yielding yellowish white compound. Repeated recrystallization through boiling with ethyl alcohol yielded β-sitosterol [7 mg, 0.0014 %, R_f = 0.21(C_6H_6); the spot was detected in iodine chamber]. Fractions eluted with hexane-chloroform (50:50-30:70) were concentrated and left overnight to yield yellowish white compound. It was washed with hexane yielding white substance which on recrystallization in hot ethyl alcohol-water solvent system, yielded diphenyl sulphone (15 mg, 0.003%). This was homogeneous on TLC in several solvent systems; spot was observed in iodine chamber [R_f = 0.65 (CH_2Cl_2); 0.28 (C_6H_6); 0.73 (EtOAc-petroleum ether 3:7)].

Brine shrimp lethality assay. Brine shrimp lethality assay was adopted following the procedure of Meyer *et al.* (1982). Brine-shrimp eggs, Red Jungle brand™ were purchased from Ocean Star International Inc., Snowville, UT 84336, USA. Eggs were incubated in artificial sea water, (which was prepared from sea salt 40 g/l, Instant Ocean Aquarium System, Inc., USA) for about 48 h kept at 27-30 °C in a water bath. After 48 h the nauplii were collected and transferred to a small beaker containing artificial sea water.

Solution was prepared by dissolving 6 mg of isolated diphenyl sulphone in chloroform (3 ml). From this solution 500, 50 and 5 µl were transferred into Eppendorf tubes (1.5 ml capacity) corresponding to 1000, 100 and 10 mg/µl, respectively. Five replicates and one control were made for each dose level. The content of each Eppendorf tube was evaporated completely at 50 °C in rotavapour at reduced pressure. Ten brine shrimps, along with sea water were transferred to each Eppendorf tube. Final volume was adjusted to 1 ml by adding more seawater. Eppendorf tubes were kept in room, illuminated with 15 w bulb, maintaining temperature 20-22 °C for 24 h. After 24 h, the number of survivors was counted and LC_{50} value was calculated by Probit analysis (Finney, 1971). LC_{50} value was expressed as mean of three independently performed experiments in mg/µl with 95% confidence interval. Berberine was used as a positive control.

Diphenyl sulphone (Fig. 1) was authenticated by EI-MS together with ^{1}H-NMR, ^{13}C-NMR, DEPT, IR, UV and melting point. El-MS showed the peaks at m/z 218, 125 and 77 due to molecular ion peak $[M]^+$, $[M-C_6H_5O]^+$ and $[C_6H_5]^+$, respectively, which are the characteristic fragments of all diaryl sulphones (Meyerson *et al.*, 1964). The ^{1}H-NMR spectrum indicated a

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complex multiplet at δ 7.48-7.60 ppm integrating six aromatic protons at *meta* and *para* positions of two phenyl rings. Similarly a multiplet occurred at δ 7.92-8.00 ppm integrating four aromatic protons at *ortho* positions of the two phenyl rings (Table 1). ^{13}C-NMR and DEPT-135 showed three aromatic olefinic carbon peaks at δ 127.64, 129.29, 133.18 and one quaternary carbon peak at δ 145.57 (Table 2) due to two identical phenyl groups. It exhibited IR absorption bands at 1376 and 1156 cm^{-1}, which are characteristics of aromatic sulphone (Bellamy, 1975). Table 1 and Table 2 show the ^{1}H-NMR and ^{13}C-NMR data. Melting point of the chemical was in very close agreement with the reported melting point (Heilbron *et al.*, 1965). Literature survey showed that diphenyl sulphone has been isolated from bacterial sources (Ellaiah *et al.*, 1998). However, its isolation from this plant is the first report in the literature.

The result of cytotoxicity of diphenyl sulphone studied by brine shrimp lethality test is presented in Table 3. It exhibited significant cytotoxicity (LC_{50} 161.69 mg/μl) against *Artemia salina* which is more than that of caffeine but lesser than berberine chloride (Meyer *et al.*, 1982). Synthetic diphenyl sulphone has been already reported as fungicidal and highly ovicidal to fruit-tree red mites (Eaton and Davies, 1950; Esrolko, 1949).

Table 1. ^{1}H-NMR ($CDCl_3$, 300 MHz) of diphenyl sulphone

Position of H	^{1}H-NMR (δ)
2, 2', 6, 6'	7.92-8.00, m
3, 4, 5, 3', 4', 5'	7.48-7.60, m

Table 2. ^{13}C-NMR ($CDCl_3$, 75 MHz) of diphenyl sulphone

C atom	^{13}C-NMR (δ)	DEPT
1, 1'	145.57	C
4, 4'	133.18	CH
3, 3', 5, 5'	129.27	CH
2, 2', 6, 6'	127.64	CH

Table 3. Brine shrimp lethality assay of diphenyl sulphone against *Artemia salina*

Compound	Concentration (μg/ml)	LC_{50} (μg/ml)	95% Confidence interval
Diphenyl sulphone	1000:100:10	161.69	193.78 - 129.60
Berberine°	1000:100:10	89.12	-
Caffeine	1000:100:10	306*	-
Berberine chloride	1000:100:10	22.5*	-

°= Positive control; * = Meyer *et al.* (1982)

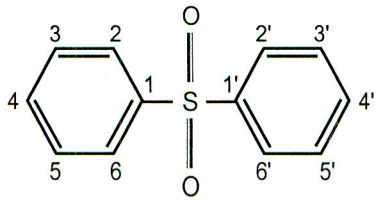

Fig. 1. Chemical structure of diphenyl sulphone.

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