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ISOLATION AND CHARACTERIZATION OF TWO STEROIDS FROM ERYTHRINA VARIEGATA L.

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ABSTRACT

Erythrina variegata L. (Fabaceae) is a thorny deciduous tree with thick clusters of scarlet or showy red flowers. In Bangladesh the plant is found throughout the country and is used as an important medicinal plant. A wide range of chemical compounds have been isolated from this plant, such as alkaloids, flavonoids, triterpenoids saponins, lectin etc. Two steroid compounds namely stigmast-4-en-3-one and stigmasta-4,22-dien-3-one were isolated as mixture from this plant by vacuum liquid chromatography (VLC) and column chromatography and the structures were elucidated based on nuclear magnetic resonance (^{1}HNMR) spectroscopic analysis.

INTRODUCTION

Steroids are used commercially as biologically active compounds, which are used in drug manufacture by the pharmaceutical industries. Steroids are pharmaceutically important in the preparation of sex hormones, corticosteroids and contraceptives. Diosgenin is an important steroidal metabolite used as a starting material for the synthesis of steroidal drugs, as it exhibits estrogenic activity. Diosgenin has indicated the effect of reducing the level of serum cholesterol. It is mainly used as the initial material for partial synthesis of oral contraceptives, sex hormones and other steroids.[1-3]

Erythrina variegata L., commonly known as tiger's claw or Indian coral tree (Bangla Name: Raktamadar, Mandar, Madar) is a species of *Erythrina* native to the tropical and subtropical regions of eastern Africa, the Indian Subcontinent, northern Australia, and the islands of

the Indian Ocean and the western Pacific Ocean east to Fiji. *E. variegata* is a thorny deciduous tree growing to 27 m (89 ft) tall and is valued as an ornamental tree.[4] Different parts of the plant have been used in traditional medicine as collyrium in opthalmia, nervine sedative, antiseptic, antiasthmatic, antiepileptic, and as an astringent. The alkaloids extracted from the leaves of *E. variegata* are reported to have anti-inflammatory and analgesic activity. Isoflavonoids isolated from *E. variegata* having antibacterial and anthelmintic activity. *E. variegata* shows several other characteristic pharmacological effects like neuromuscular blocking and hydrocholeretic, which are consistent with the reported uses of the plant extracts in the indigenous system of medicine.[5] The aim of this investigation is to isolate different secondary metabolites from this plant.

MATERIALS AND METHODS

General experimental procedures: Compounds were detected with vanillin H_2SO_4 spray reagent. Column chromatography was performed using silica gel. ^{1}H NMR spectra were recorded in CDCl3 (δ values were reported in reference to CHCl3 at 7.25 ppm) on a Bruker Avance400 MHz Ultrashield NMR Spectrophotometer.

Plant material

Stem Bark of *Erythrina variegata* L was collected from the campus of University of Dhaka in December 2015. Plant material was taxonomically identified by the taxonomist Shah Mohammad Ahsan Habib, Senior Herberium Technitian, Bangladesh National Herbarium, Dhaka, where a voucher specimen (accession number DACB No.46874) has been deposited for future reference.

Extraction and Isolation

The stem bark of *E. variegata* was sun dried and then ground into course powder using a grinding machine and the powder plant material (500 gm) of *E. variegata* was soaked in 1 liter of methanol for 20 days and then filtered through a cotton plug followed by whatman filter paper number 1. The extract was concentrated at reduced pressure with a vacuum rotary evaporator at 40°C. An aliquot of the crude methanolic extract (25.6 g) was fractionated by vacuum liquid chromatographic (VLC) technique using silica gel 60H and petroleum ether, petroleum ether-dichloromethane, dichloromethane-ethyl acetate, ethyl acetate and ethyl acetate -methanol in increasing order of polarity and thus 36 VLC fractions were obtained. VLC fraction 13 was further fractionated by column chromatography and from the column

fraction 30-33, a colourless crystal was obtained which was further purified by recrystallization process.

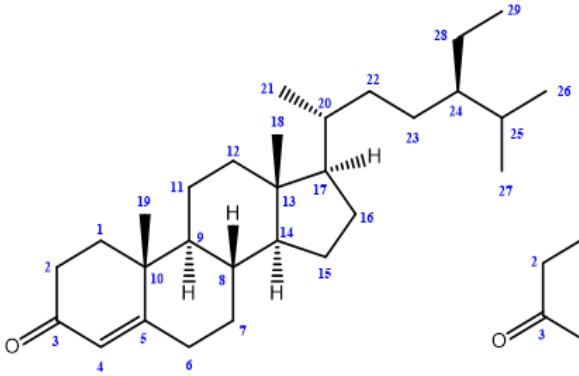
RESULTS AND DISCUSSION

Chromatographic separation and purification of the methanol soluble extract of the stem bark of *E. variegata* provided two steroid compounds (1-2), the structures of which were elucidated by ^{1}H NMR analysis.

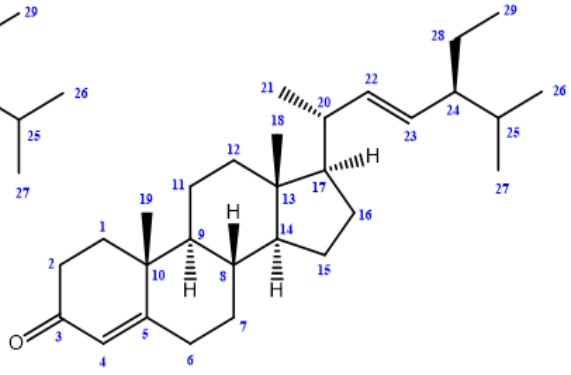
Compound 1 and compound 2 were isolated from VLC fraction 13. The compounds were found as mixture of colourless crystals and produced purple color when sprayed with vanillin in sulphuric acid reagent, followed by heating for 5 minutes. The compounds were appeared as a single spot on a TLC plate and therefore could not be separated from each other.

The ^{1}H NMR spectrum of compound 1 (Table1) showed resonances for six methyl groups at δ 0.73s, 0.83 *d* (*J* = 7.2 Hz), 0.86 *d* (*J* = 7.2 Hz), 0.87 *t* (*J* = 7.2 Hz), 0.94 *d* (*J* = 6.6 Hz) and 1.20 s assignable to H-18, H-27, H-26, H-29, H-21 and H-19 respectively. An olefinic proton appeared as a sharp singlet at δ 5.74 assignable to H-4. The ^{1}H NMR spectrum were found similar to those reported for sitosta-4-en-3-one.[6] Thus compound 1 was identified as stigmast-4-en-3-one.

The remaining signals of the^{1}H NMR spectrum (Table 1) include two methyl singlets at 0.75 and 1.20, three methyl doublets, 0.83 (*J* = 7.2 Hz), 0.86 (*J* = 7.2Hz) and 1.04 (*J* = 6.8 Hz), a methyl triplet at 0.87 (*J* = 7.2 Hz) and an olefinic proton singlet at δ 5.74. In addition, the spectrum displayed two *trans* olefinic protons, as indicated by the large coupling constant of 15.2 Hz, resonated at 5.04 dd and 5.17 dd ((*J* = 15.2, 8.4 Hz, each). On this basis, compound 2 was identified as stigmasta 4-22-dien-3-one. All these ^{1}HNMR data were found to be in close agreement with those reported for stigmasta-4, 22-dien-3-one.[6]



Stigmast-3-en-4-one



Stigmasta-4,22-dien-3-one

Table 1: NMR spectroscopic data (400 MHz, $CDCl_3$) for compounds 1 and 2.

Position	Compound 1	Stigmast-4-en-3-one[6]	Compound 2	Stigmasta-4,22-di en-3-one[6]
	δ_H	δ_H	δ_H	δ_H
H-4	5.74 1H s	5.72 1H s	5.74 1H s	5.72 1H s
H-18	0.73 3H s	0.71 3H s	0.75 3H s	0.73 3H s
H-19	1.20 3H s	1.18 3H s	1.20 3H s	1.18 3H s
H-21	0.94 3H d (*J* = 6.4 Hz)	0.92 3H d (*J* = 6.5 Hz)	1.04 3H d (*J* = 6.8 Hz)	1.02 3H d (*J* = 7.5 Hz)
H-22	---	---	5.17 1H dd (*J* = 15.2, 8.4 Hz)	5.15 1H dd (*J* = 15.5, 9.0 Hz
H-23	---	---	5.04 1H dd (*J* = 15.2, 8.4 Hz)	5.03 1H dd (*J* = 15.5, 9.0 Hz
H-26	0.86 3H d (*J* = 7.2 Hz)	0.84 3H d (*J* = 6.8 Hz)	0.83 3H d (*J* = 7.2 Hz)	0.80 3H d (*J* = 6.0 Hz)
H-27	0.83 3H d (*J* = 7.2 Hz)	0.82 3H d (*J* = 6.8 Hz)	0.86 3H d (*J* = 7.2 Hz)	0.85 3H d (*J* = 6.0 Hz)
H-29	0.87 3H t (*J* = 7.2 Hz)	0.85 3H m	0.83 3H t (*J* = 6.4 Hz)	0.81 3H m

CONCLUSION

Erythrina variegata L. has many uses as a a medicine and contains many important secondary metabolites. In this investigation two steroid compounds were isolated from *E. variegata* which were stigmast-4-en-3-one and stigmasta-4, 22-dien-3-one. Further work is to be carried out to explore another new method to separate the compounds from the mixture.

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