EXTRACTION, ISOLATION AND IDENTIFICATION OF PHYTOCONSTITUENTS FROM THE LEAVES OF *CORDIA DICHOTOMA* G.FORST.

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Abstract

The present study was the phytochemical analysis of the leaves from *Cordia dichotoma* G. Forst. (Boraginaceae). It was collected from Loilem Township, Southern Shan State. *Cordia* is one of most important genus of family Boraginaceae and involves a wide range of therapeutic uses in traditional medicine. Cool extraction method was followed to prepared the extracts, followed by standard phytochemical methods to identify the phytoconstituents present in it. Thin layer chromatography (TLC) and Column chromatography studies were then done for the confirmation and quantification of phytoconstituents. The defatted methanol extract (8 g) was fractionated by Column Chromatography method. The isolated compounds were identified by TLC, R_f value, melting point, UV and FT IR spectroscopic analysis. It was found that the isolated compound A, steroidal glycoside (R_f value 0.56, yield 0.032 %) was obtained from Fraction I, compound B, lupeol (melting point 215°C, R_f value 0.48, yield 0.005%) from Fraction IV and compound C, quercetin (melting point 321°C, R_f value 0.38, yield 0.014 %) from Fraction V. The presence of steroidal glycoside, lupeol and quercetin are indicative of potential for medicinal use of this plant.

Introduction

Plants are very important source of potentially useful bioactive principles for the development of new chemotherapeutic agents. *Cordia dichotoma* G.Forst., commonly known as Thanatphet or Thanat in Myanmar, belongs to the family Boraginaceae, subfamily Cordioideae The *Cordia* genus comprises more than 300 species, mostly evergreen trees and shrubs distributed widely in the tropical regions.

In Myanmar, *Cordia dichotoma* G. Forst.is especially found in southern Shan State, Kachin State, Kayah State, Mandalay Division, Yangon Division. Leaves, fruit, bark and seed of *Cordia dichotomas* G.Forst. are extensively used in traditional medicine for antimicrobial, anti-inflammatory, anthelmintic, analgesic and diuretic purposes and for treating digestive system, respiratory, urogenital, cardiac, vascular and blood disorders (Matias *et al*., 2015; Kumari *et al*., 2016).

Various secondary metabolites like alkaloids, phenolic compound, tannins, flavonoids, steroids and terpenoids were isolated from the leaves of *Cordia* dichotoma G.Forst.plants. Srivastava (1979) reported that α-amyrins, betulin, lupeol, quercetin, β- sitosterol and quinone are mainly present in leaves and seeds of *Cordia dichotoma* G.Forst. Thus, the aim of this study is to extract and isolate the organic compounds from the leaves of *Cordia dichotoma* G. Forst. and to analyses that isolated compound by modern Spectroscopic Techniques.

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Materials and Methods

Collection and preparation of sample

The sample plant *Cordia dichotoma* G. Forst. was collected from Loilem Township, Southern Shan State during the months of April to July, 2018. The sample leaves were washed with water to eliminate dust and cut into small pieces and air dried in room temperature for several days. After being completely dried, they were pulverized by grinder machine to get powder and stored in airtight containers to prevent it from moisture and air-borne contamination (Fig 1).

Figure 1 Habit of *Cordia dichotama* G. Forst.

Preparation of methanol extract from leaves of *Cordia dichotoma* G. Forst.

The air dried leaves powdered sample of *Cordia dichotama* G. Forst. (200 g) was macerated with 2 liter of Petroleum ether (60-80°C) for 4 weeks and filtered. Defatted marc was macerated with 2.5 liter of methanol for 4 weeks and filtered. This filtrate was concentrated on water bath. Finally, the defatted methanol crude extract (8.95 g) was obtained from dried powdered (200g) of *Cordia dichotoma* G. Forst. (Figure.2).

Isolation of compounds A, B and C from methanolic extract of leaves of *Cordia dichotoma* G. Forst.

Isolation of phytoconstituents by using Column Chromatographic method and identified by R_f value, melting point, UV and FT IR spectroscopic method.

Determination of (R_f) values of isolated compounds (Sherma & Fried, 2005)

$$R_f \text{ (Retardation factor)} = \frac{\text{Distance of chromatographic spot center from the start}}{\text{Distance travelled by the solvent from the start}}$$

Ultraviolet (UV) spectroscopic study of isolated compounds

The ultraviolet spectrums of isolated compounds were determined by UV-1800 Spectrophotometer at Department of Chemistry, University of Yangon. (Figure.7 and 8)

Infra-red (IR) spectroscopic study of isolated compounds

The infra-red spectrums of isolated compounds were determined by using Perkin Elmer Spectrum Two spectrometer at Department of Chemistry, University of Yangon. (Figure.9, 10 and 11)

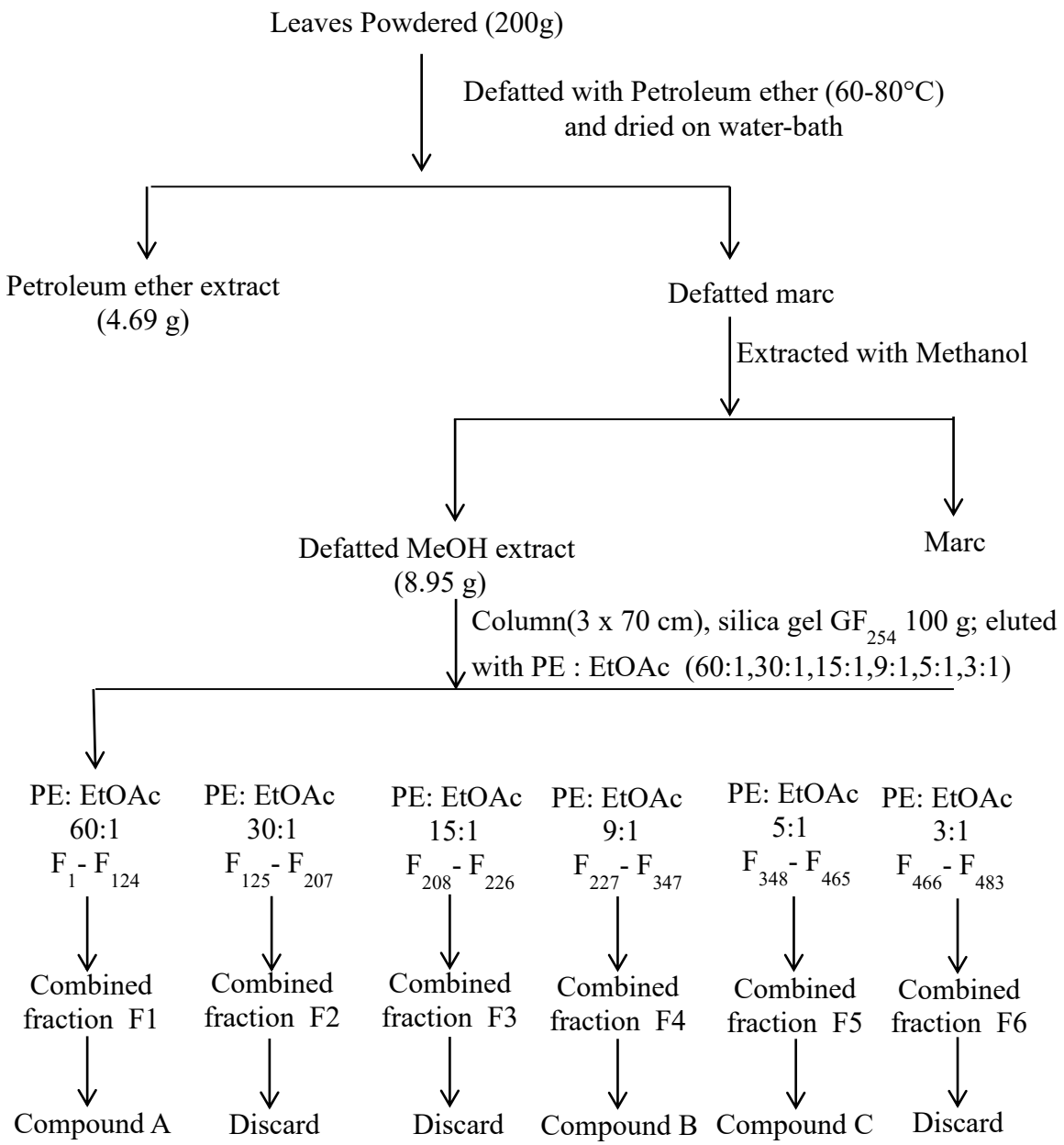


Figure 2 Flow diagram of extraction and isolation of compound A, B and C from the powdered leaves of *Cordia dichotoma* G.Forst.

Results

Isolation, Identification and characterization of phytoconstituents from Cordia dichotoma G.Forst.

The isolation of phytoconstituents from the leaves of *Cordia dichotoma* G.Forst. by using Column chromatography separation method. The three known compounds such as the compound A (light yellow oil), Steroidal glycoside (0.032 %); compound B (colorless needle shape), Lupeol (0.005 %) and compound C (yellow crystal), Quercetin (0.014 %) were isolated. These isolated compounds were identified by R_f value, Thin Layer Chromatography (TLC), physico-chemical characteristics of isolated compounds, UV and FT IR Spectroscopic analysis. The results were shown in Fig (4, 5, 6).

Identification of isolated compound A

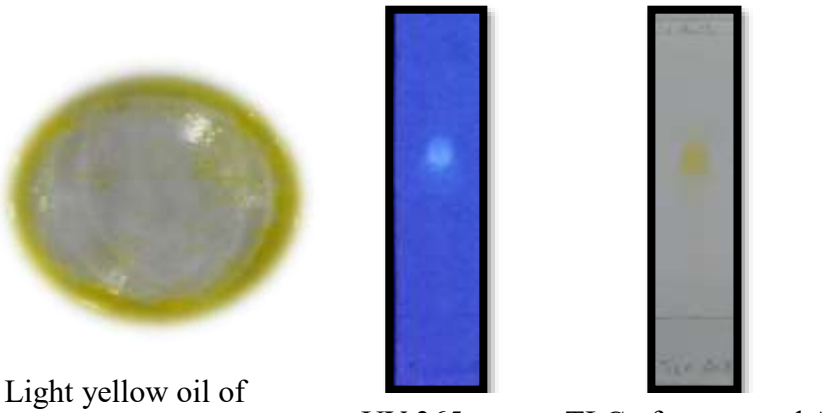
The isolated compound A (oil, 0.032 % yield) was fractioned from fraction F1 (petroleum ether: ethyl acetate, 30:1) was done by using column chromatography method. According the results of physico-chemical characters, Compound A was light yellow color. After Libermann - Burchard test, green colour of compound A was observed. It is UV active (UV 365nm). The single spot was visualized on TLC plate by spraying with 5% H_2SO_4 and vanillin. The compound A may be steroid compound. The R_f value of compound A was 0.56 which was in similar steroidal glycoside (Okwu, & Ohenhen, 2010). Therefore compound A may be steroidal glycoside. The results were shown in Figure 4.

Identification of isolated compound B

The isolated compound B (colorless needle-shape, 0.005 % yield) was fractioned from fraction F4 (petroleum ether: ethyl acetate, 9:1). According the results of physic-chemical characters, Compound B was pink colour by using Libermann-Burchard test. It is UV inactive (UV -254nm & 356nm). The single spot was visualized on TLC plate by spraying with 5% H_2SO_4 and vanillin. The compound B may be terpenoid compound. The R_f value of compound B was 0.48 and melting point 215 °C which was in agreement lupeol (Merck Index, 2001). Therefore, compound B may be lupeol. The results were shown in Figure 5.

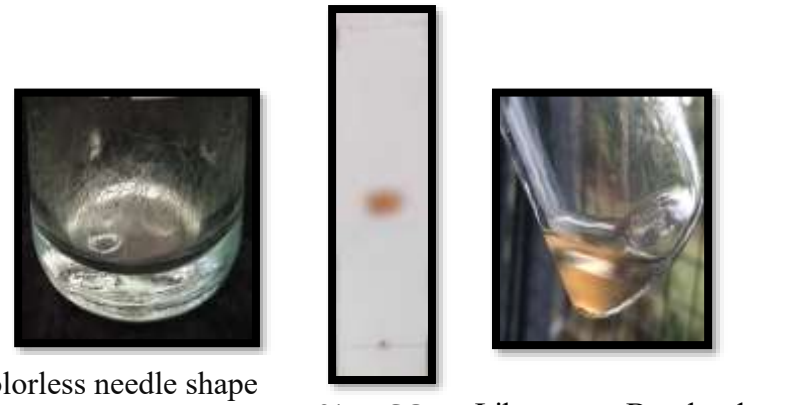
Identification of isolated compound C

The isolated compound C (Yellow crystal shape, 0.014 % yield) was fractioned from fraction F5 by column chromatography with petroleum ether: ethyl acetate (5:1). According the results of physico-chemical characters, Compound C was yellow colour when treated with magnesium ribbon and concentrated hydrochloric acid. So the compound may be flavonoid. The R_f value of compound C was 0.38, melting point 321 C°, which was in agreement with quercetin (Abeer, 2011). Therefore compound C may be quercetin. The results were shown in Figure 6.



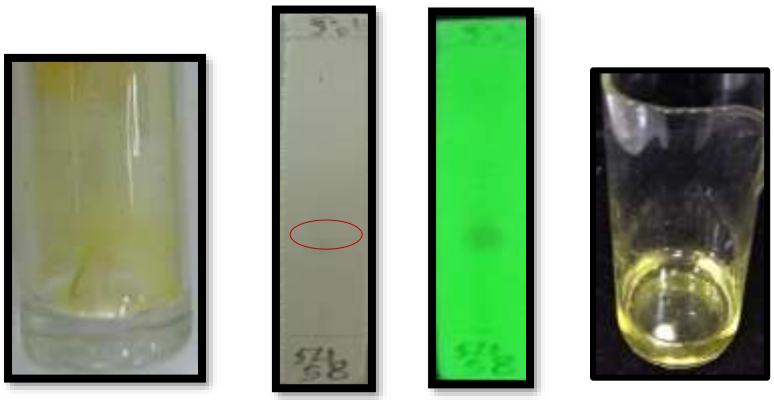
Light yellow oil of compound A | UV-365 nm | TLC of compound A

Figure 4 TLC and crystal shape of isolated compound A



Colorless needle shape crystal of compound B | 5% H_2SO_4 | Libermann-Burchard test

Figure 5 TLC and crystal shape of Isolated Compound (B)

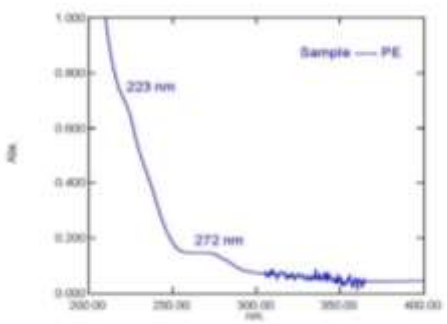


Yellow crystal of compound C | Visible | UV-256 nm | Flavonoid Test

Figure 6 TLC and crystal shape of Isolated Compound (C)

Ultra violet spectroscopic study

The absorption maximum wave length of isolated compound A was found at 223, 272 nm indicating the presence of conjugated double bond. Compound C in methanol observed two absorption maxima at 256 nm (band II) and 373 nm (band I) agreements with flavonoid. By adding of NaOH, band II shifted 285 nm that indicated the presence of 7-OH and band I shifted 421 nm which show the present of 4′ OH. The UV spectra of these compounds were shown in Figure 7 and 8.



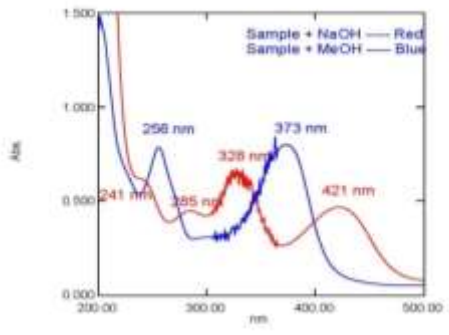


Figure 7 UV spectrum of isolated compound A **Figure 8** UV spectrum of isolated compound C

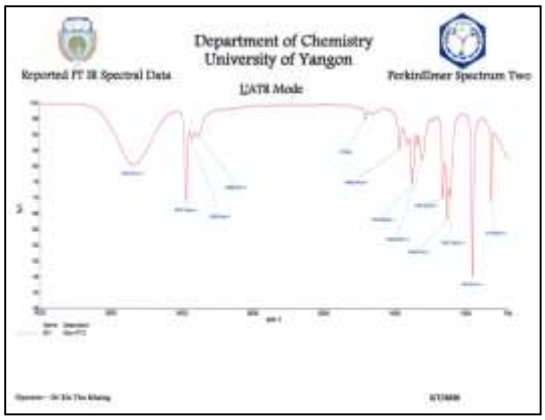


Figure 9 FT IR spectrum of Compound A

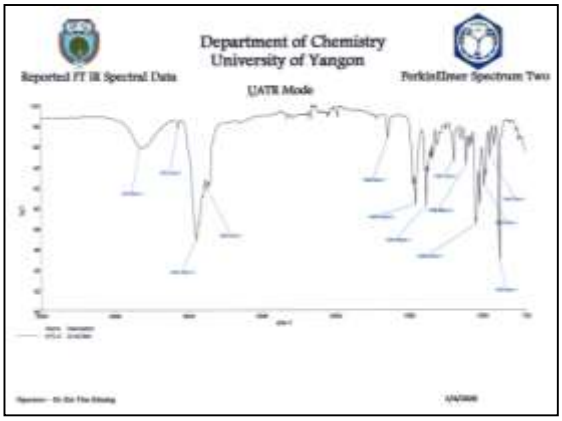


Figure10 FT IR spectrum of Compound B

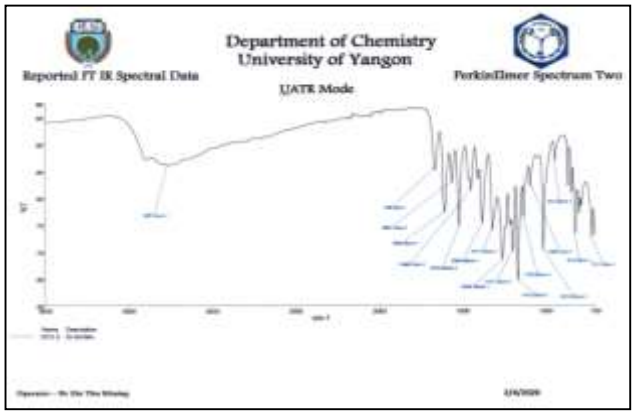


Figure 11 FT IR spectrum of Compound C

Identification of fourier transform infra- red spectrum FT IR of compound A

IR spectrum of isolated compound (A) indicated the presence of OH stretching of alcoholic group (3342 cm^{-1}), C-H stretching of CH_2 group (2970 cm^{-1}), C-H stretching asymmetric of CH_2 and CH_3 group (2970 cm^{-1}), C-H stretching symmetric of CH_2 and CH_3 group (2880 cm^{-1}), C=O stretching of carbonyl group (1708 cm^{-1}), C=C stretching of aromatic ring group (1466 cm^{-1}), C-O stretching of cyclic alcohol group (1378 cm^{-1}), C-O stretching of ether group (1048 cm^{-1}). The FT IR spectrum of the compound (A) was shown in Figure.9. (Okwu & Ohenhen, 2000)

Identification of fourier transform infra- red spectrum FT IR of compound B

IR spectrum of isolated compound (B) indicated the presence of OH stretching of hydroxyl group (3315 cm^{-1}), = C-H stretching of vinylidene group (3070cm^{-1}), C-H stretching asymmetric of CH_2 and CH_3 group (2944 cm^{-1}), C-H stretching symmetric of CH_2 and CH_3 group (2872 cm^{-1}), C=C stretching of alkene group (1636 cm^{-1}), C-H bending of CH_2 and CH_3 group (1452 cm^{-1}), C-H bending of gem dimethyl group (1379 cm^{-1}),), CH-OH stretching of cyclic alcohol (1042 cm^{-1}). The FT IR spectrum of the compound (B) was shown in Figure 10. (Herbone, 1984)

Identification of Fourier transform infra- red spectrum FT IR of compound (C)

IR spectrum of isolated compound (C) indicated the presence of OH stretching of phenolic O-H group (3257 cm^{-1}), C=O stretching of cyclic conjugated system (1667cm^{-1}), C-O stretching of aromatic ring (1608 cm^{-1}), C=O stretching of aromatic ring (1519 cm^{-1}), O-H banding of phenol group (1380 cm^{-1}), C-O-C stretching of ether group (1259 cm^{-1}), C-O stretching of phenol group (1197 cm^{-1}). The FT IR spectrum of the compound (B) was shown in Figure 11. (Bharathi *et al*., 2016)

Discussion and Conclusion

In this research work, extraction, isolation and identification of phytoconstituents from the leaves of *Cordia dichotoma* G.Forst. has been investigated. These organic compounds were obtained from methanol extracts of leaves of *Cordia dichotoma* G. Forst. by Column Chromatographic method.

The isolated compound A with yellow oil (yield - 0.034%) R_f value 0.56. The absorption maximum wave length of isolated compound A was found at 223, 272 nm indicating the presence of conjugated double bond. So, compound A may be steroid compound. According to FT-IR spectrum of compound A, the absorption band at 3342 cm^{-1} indicating the presence of OH-stretching alcohol group, aliphatic CH_2 stretching band appeared at 2970cm^{-1}, the absorption band at1708 cm^{-1} indicating the presence of C=O stretching of carbonyl group, the absorption band at 1048cm^{-1} indicating the presence of C- O stretching of ether group. Therefore, compound "A" may be assigned as steroidal glycoside (Merck index, 2001; Okwa & Ohenhen, 2010).

The melting point of compound B was 215 °C and R_f value was 0.48 and UV inactive. According to physico-chemical tests, isolated compound B was a terpenoid compound. The melting point of compound B is coincident with that of literature lupeol (Herbone, 1984; Merck index, 2001). According to FT IR spectrum of isolated compound B, O-H stretching vibration band appear at 3257 cm^{-1}, the C-H stretching band appeared at 3070 cm^{-1} indicating the presence of vinylidene group, CH stretching band appeared at 2944 cm^{-1} and 2872 cm^{-1} represent the present of CH_2 and CH_3 groups. The absorption band at 1636 cm^{-1} due to C=C stretching indicated the present of double bond. According to the results obtained from FT IR spectral data and melting point, the isolated compound B may be assigned as lupeol(Merck index, 2001).

The isolated compound C was obtained as yellow crystals, melting point 321 °C, R_f value 0.38. The melting point of compound C is similar with that of literature quercetin (Abeer, 2011). The UV spectrum of compound C in methanol observed two absorption maxima at 256 nm (band II) and 373 nm (band I) agreements with flavonoid. By adding of NaOH, band II shifted 285 nm that indicated the presence of 7-OH and band I shifted 421 nm which show the present of 4′ OH (Jain *et al*., 2011) In FT IR analysis, the absorption band occurred at 3257 cm^{-1} due to the OH stretching of phenolic group. The C=O stretching of cyclic conjugated system appeared at 1667cm^{1}. The absorption band at 1608 cm^{-1} and 1519 cm^{-1} were assigned for C=C stretching of aromatic ring. The absorption band at 1380 cm^{-1} appeared due to OH bending of phenolic group and the band at 1259 cm^{-1} associated with C-O-C stretching of ether group and the band at 1197 cm^{-1} was C-O stretching of phenol group. According to melting point, chemical test, UV and FT IR spectral data, the compound C may be assigned as quercetin (Bharathi *et al*., 2016).

It was concluded that, *Cordia dichotoma* G. Forst. is an important therapeutic medicinal plant with varied pharmacological spectrum. The result revealed that *Cordia dichotoma* G. Forst. showed the presence of bioactive compounds (a steroid compound, lupeol and quercetin) which are responsible for varied pharmacological and therapeutic property. The evaluation needs to be carried out on *Cordia dichotoma* G.Forst. in dorder to use the plant in various clinical applications.

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