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Research Article

Gibberellic Acid and Potassium Nitrate Promote Seed Germination and Growth of Grey-leaved Saucer-berry (*Cordia Sinensis* Lam.) Seedlings

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ABSTRACT

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However, maximum plant height was observed under GA_3 500 ppm and KNO_3 (1%) treatment. In conclusion, the combination treatment of GA_3 250 ppm and KNO_3 1% is an effective and practical method for improving C. sinensis seed germination.

KEYWORDS: Cordia sinensis | gibberellic acid | potassium nitrate | seed germination

Introduction

Cordia sinensis Lam. (Cordia gharaf (Forssk.) Ehrenb. ex Asch.) is a multi-stemmed tree, growing up to 8 to 12 m height. Cordia sinessis is a potential underutilized plant which is also known as Gondi, narrow-leaved sebastian or Greyleaved saucer-berry and belongs to the family Boraginaceae. The genus Cordia consists of about 250 species and the majority of species are tree or shrub (Barroso and Oliveira, 2009). It is well-adapted plant species in arid to semiarid regions of West Africa to East to the Middle East, India, Sri Lanka, and Pakistan due to its various adaptive features e.g. strong tap root system, waxy leaves, hairiness, sunken and covered stomata in leaves, water binding mechanism, moderate tolerance to salinity and alkalinity. These traits make it hardier than Cordia myxa, a popular fruit plant of the Boraginaceae family (Meghwal et al., 2014). The fruits of the C. sinensis Lam. are consumed by local inhabitants. The leaves provide the protein (13.12%) rich fodder to cattle (Kuria et al., 2005). Various parts of the plant have ethnomedicinal value and are traditionally used to cure different human and livestock ailments (Gumgumjee and Hajar, 2015). Farmers of arid regions grow C. ot and dry wind (M ies for domesti f C. sinensis was abo on of the Gujarat nensis was distri is reported to be Haryana) (Singh, ut germina ous germina ieri reveals conflicti tification

treatment with gibberellic acid (GA_3) (250, 500 mg L^{-1}) promotes germination significantly (Meghwal, 2006)) Similarly, about 50–60% seed germination could be obtained with fresh seeds of C. sinensis, but the germination percentage is low and not uniform (Maundu et al., 2005; Meghwal et al., 2014). However, another study in Cordia biosseri showed high percentage of seed dormancy in the newly produced seeds (Fulbright, 1992; Schuch et al., 2001). Both GA_3 and KNO_3 stimulate the seed germination of dormant seeds of various plant species (Koyuncu 2005; Cárdenas et al., 2013; Mello et al., 2009). However, the studies carried out by Ding et al. (2007), Gao et al. (2011), Cárdenas et al. (2013) observed the effect of GA_3 and KNO_3 on seed germination of other species but very few on C. sinensis seed germination.

The proper seedling growth of germinated seeds is the most important factor for successful sapling production under nursery conditions. The earlier studies showed the variable effect of GA_3 and KNO_3 on growth traits of various plants. Application of GA_3 and KNO_3 promotes overall plant growth in Brassica oleracea Capitata (Majumdar, 2013), Cucumis sativus (Pal et al., 2016), and Solanum lycopersicum (Balaguera-Lopez et al., 2009). GA_3 (250 mg L^{-1}) treatment resulted in maximum seed germination (98.75%), subsequent shoot length, root length, leaf area, shoot, and root dry weight (Shabaq, 2013). Similar growth promotion of seedlings was also reported under the GA_3 treated seeds of Citrus limon (Dzayi and Rahman, 2010), Nothapodytes nimmoniana (Patil et al., 2015), and Citrus aurantifolia (Jaiswal et al., 2018). Lay et al. (2015), found GA_3 and KNO_3 presowing treatment to seeds of papaya improves the seedling growth, fresh and dry weight of seedlings. However, very few studies are available with quantitative data on the subsequent growth of treated plants. The C. sinensis is very importa odder, fruits, and mec r and erratic g the seed germina on for the Cordia s

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carried out under the shade net. Cordia sinensis fruits were collected from the native population in the Kachchh region of Gujarat (22°84′624 – 23°56′288 N° latitude and 68°95′730 – 70°73′100 E° longitude). Fresh seeds were extracted from the ripened fruits. The mucilaginous pulp was removed from the seeds by rubbing the seeds with sandy soil followed by washing with tap water. Air dried, cleaned seeds were stored in the paper bag for 2 weekss at the room temperature before use.

Experiment 1. Chemical Treatment of Seeds

Before the seed treatment, seeds were disinfected with fungicide (Carbendazim 2%) for 20–30 minutes and rinsed three times with running tap water. For the treatments, seeds were divided into four groups, the total no. of treatment combinations were 16, four levels of each of GA_3 concentration (0, 250, 500, and 1000 ppm) and four KNO_3 concentrations (0%, 0.25%, 0.5%, and 1% KNO_3) purchased from Sigma Aldrich (90% gibberellin A_3 basis) Loba Chemie (99% purity), respectively and were evaluated with 3 replications 1) seeds of first group were transferred into the beaker filled with distilled water and left for 24 h; 2) seeds of second group were soaked in 250, 500, and 1000 mg L^{-1} (w/v) GA_3 solution for 24 h; 3) similarly, seeds of the third group were soaked in 0.25%, 0.5%, and 1% KNO_3 solution for 24 h; 4) the seeds of the fourth group were treated with the aqueous solution supplemented different combinations of GA_3 and KNO_3 (Table 1).

Table 1. Test design of chemicals (KNO_3, GA_3) and combinations of KNO_3 and GA_3 treatments

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germination was recorded at the weekly interval by unfolding the blotting paper up to 30 days of sowing after which there was no further germination noticed. The germination percentage was calculated as follows (Gashi et al., 2012)

2G2%2=a×100/N

Where G = germination percentage, a = the total no. of germinated seeds till 30 days of sowing and N = the total no. of seeds sown.

While, mean germination time was estimated using the following method (Moradi et al., 2008).

MGT = ΣDn/Σn

Where n = number of seeds which were germinated on day D,

D = number of days taken for seed germination.

Experiment II. Seedling Growth at Net House

After the 30-day, the germinated plantlets of C. sinensis were shifted from laboratory to green shade net (50% shade and 1.5 mm thickness) conditions. The plantlets were established in the black-colored nursery poly bags (20 × 10 cm) and filled with sterilized soil (sand: soil: farmyardd manner – 2:2:1) potting mixture. The average temperature and relative humidity under shade net house during experiment were about 30°C and 74%. Different plant characteristics like plant height (cm), stem thickness (mm), no. of leaves per plant were measured at 30, 90, 180, 270, and 360 day y weight (g), leaf timated at the end easured using a ording leaf dry weig constant weigh re estim tergent solution, nples were dried in ight and ground a e flame photome nt was done

Statistical Analysis

The randomized complete block design (RCBD) was used for conducting the experiment in laboratory and shade net. The critical differences (CD) at $P < .05$ and $P < .01$ level of probability were determined after analysis of variance (ANOVA) for all treatments. The experiment was laid out with 16 treatments including control with three replications and 60 seeds per replicate were used. The mean and analysis of variance were performed to determine significant differences among treatments ($P < .05$) using the Statistical Software Package for Agricultural Research Workers (Sheoran et al., 1998).

Result and Discussion

Experiment 1. Chemical Treatment of Seeds

Mean Germination Time (MGT) and Germination Percentage (GP)

The different concentrations of GA_3 and KNO_3 significantly fasten the seed germination as compared to the germination percentage of control seeds. The KNO_3 (0.5%) treatment significantly shortens the germination initiation duration (4 days), compared to other single KNO_3 applications. Whereas the synergistic effect of $GA_3 \times KNO_3$ treatments was observed on the shortening of germination time. The seeds treated with aqueous solution supplemented with GA_3 250 mg L^{-1} and 1% KNO_3, took minimum time for germination initiation (4.33 ± 0.33 days), which was about 1 week less tha maximum time for .25% and 1.0% co

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highly significant difference was noted between seeds treated with a different gibberellic acid concentration (250, 500, and 1000 mg L^{-1} GA_3) and with a combination of the different concentration of potassium nitrate (0.25%, 0.5%, and 1%) and untreated seeds (control). The maximum final germination percentage was noted in the seeds treated with 250 mg L^{-1} GA_3 × 1% KNO_3 (79.47%) and 500 mg L^{-1} GA_3 × 0.25% KNO_3 (67.17%), as compared to the germination percentage of control (21.97%). In contrast, when seeds treated with a higher GA_3 concentration along with higher KNO_3 treatment, the final germination percentage reduced drastically. Analysis of data revealed that both single factors (GA_3 and KNO_3 concentration) affected seed germination percentages ($p \leq 0.05$). However, higher germination percentage was reported under the 0.5% KNO_3 treatment, which was higher than all other single treatments. However, GA_3, treatment singly did not significantly increase the final seed germination percentage. Similar study conducted in Cordia biosseri reveals a high percentage of dormant seeds and GA_3 treatment, breaks the seed dormancy (Fulbright, 1992; Schuch et al., 2001). Similarly, Meghwal (2007) noted 20–30% seed germination in Cordia myxa and up to 60% seed germination in Cordia gharaf with freshly extracted seeds and seed treatment with GA_3 and mechanical scarification significantly improve (66.66%) germination of species. On the contrary, Maundu et al. (2005) observed that, seed treatment of fresh seeds of Cordia sinensis generally not necessary but, stored seeds need to be soaked with warm water (40°C) until water is cool. However, Acharya and Purohit (1999) found that GA_3 promoted germination of seeds in Cordia gharaf to the maximum extent of 81%. Waman et al. (2017) recommended that presowing treatments with KNO_3 and GA_3 were effective to produce the healthy seedlings of Semeca overall percenta promotes the germ s (Ding et al., 2007 et al., 2018) and Elae increases the germ emurus spec arpa (Cárden ts of Cárdena irectly proportio However, at higher G ecreased (Cárden $^{-1}$) promotes

nimmoniana (Patil et al., 2015) and Citrus aurantifolia (Jaiswal et al., 2018). Seed germination was improved due to gibberellic acid (GA) treatment possibly through increased synthesis of hydrolytic enzymes, which were further transported to endosperm. The enzymes break down the stored food to supply the energy required for germination (Chen et al., 2008; Varner, 1965). GA_3 may promote cytokinin action and counteracts the germination inhibitor (ABA) which ultimately stimulate the biochemical processes of seed germination (Chen et al., 2008; Cetinbas and Koyuncu, 2006; Tuan et al., 2018). Potassium nitrate improves the seed germination percentage in the various plants species (Duermeyer et al., 2018; Keskiner and Tuncer, 2019). A study on the germination of mountain ash seeds showed that germination of the species was affected by both the concentration of KNO_3 and the time of KNO_3 exposure (Bian et al., 2013). Similarly, Gupta et al. (2011) reported that treatment of Hippophae salicifolia seeds with 0.1% KNO_3 pretreatment resulted in 96% seed germination. However, a higher concentration of KNO_3 negatively affects seed germination. A similar trend of reduction of seed germination on the application of higher KNO_3 concentration was reported in Salvia cyanescens (Yücel and Yilmaz, 2009) and Gladiolus alatus (Ramzan et al., 2010).

Table 3. Mean of germination, growth (MGT, GP, LA, LFW, LDW) and seedling nutrients (NPK and Na) of Cordia sinensis under various levels of GA_3 (in ppm) and KNO_3 (in %)

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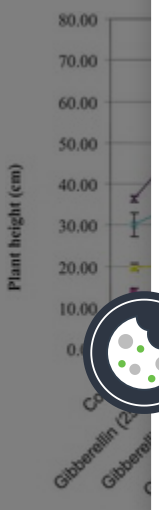
germination (66.66%). However, KNO_3 did not influence the seed germination, irrespective of treatment duration and chemical concentration. Gashi et al. (2012) found the higher germination percentage (92.26%) in the Ramonda species' seeds treated with 1000 ppm GA_3 + 0.3% KNO_3. Treatment of Sorghum bicolor seeds with GA_3 (100 ppm) and KNO_3 (1%) resulted in increase of germination percentage to a higher extent (94%) (Shanmugavalli et al., 2007). Similar synergistic results of KNO_3 (1%) and GA_3 (500 ppm) seed treatments were reported by Dewir et al. (2011) in Sabal palmetto. Whereas, soaking cracked seeds of Elaeocarpus prunifolius in the solution of GA_3 and KNO_3 accelerate the germination rate (Iralu and Upadhaya, 2018).

Experiment 2. Subsequent Quantification of Growth

Plant Height and Collar Diameter

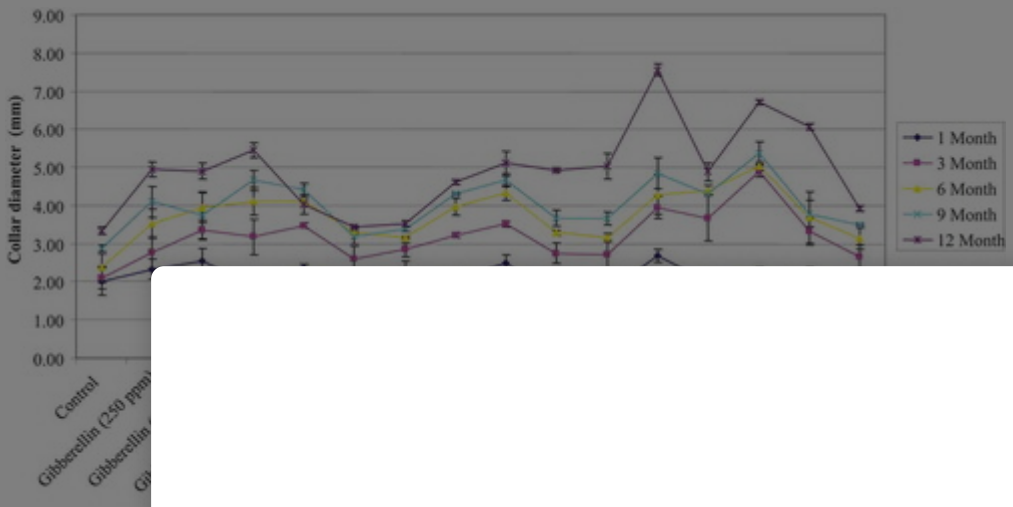
Application of GA_3 × KNO_3 singly or in combination, significantly ($p = .05$) affected the plant height (Figure 1). However, plant height under different single KNO_3 applications differed significantly. Lower KNO_3 application (0.25% conc.) tends to increase plant height at par to GA_3 500 mg L^{-1}, but at higher KNO_3 application (1%), there was a reduction in the plant height. The longest plants were observed from the GA_3 (500 mg L^{-1}) and KNO_3 (1%) applications, followed by combined application of 500 mg L^{-1} GA_3 and 0.5% KNO_3. Whereas, the minimum increase in the plant height was seen when plants seeds soaked in the GA_3 1000 mg L^{-1} and KNO_3 1% solutions for 24 h.

Figure 1. Plant height of Cordia sinensis, under various levels of GA_3 (in ppm) and KNO_3 (in %) seed treatment, recorded at three month interval



Data presented in Figure 2 illustrated the effect of GA_3 and KNO_3 on the collar diameter of C. sinensis. The significant difference was ascertained for C. sinensis between seeds treated with GA_3, KNO_3 and H_2O (control). All GA_3 and KNO_3 applications had a positive effect on collar diameter. However, single GA_3 applications were more effective than different single KNO_3 application. Moreover, integrated application of GA_3 and KNO_3 resulted pronounced improvement in collar diameter of plantlets. The maximum increase in the collar diameter in one-year-old plants was observed with the GA_3 500 mg L^{-1} and KNO_3 0.5% treatment followed by GA_3 1000 mg L^{-1} and KNO_3 0.25% treatment. On the contrary, the minimum collar diameter was recorded in the plants of control group. The presowing treatment with lower concentration of GA_3 and KNO_3 improved the seedling height significantly however, reduction in the plant height was observed under the higher concentration of GA_3 and KNO_3. Treatment of Eriobotrya japonica seeds with lower concentration of GA_3 (250 mg L^{-1}) resulted maximum collar diameter and plant height comparing to control (Shabaq, 2013). Similarly, maximum plant height and stem diameter of Citrus aurantifolia plantlets were observed with GA_3 (200 mg L^{-1}) seed treatment (Meshram et al., 2015).

Figure 2. Collar diameter of Cordia sinensis, under various levels of GA_3 (in ppm) and KNO_3 (in %) seed treatment, recorded at three month interval



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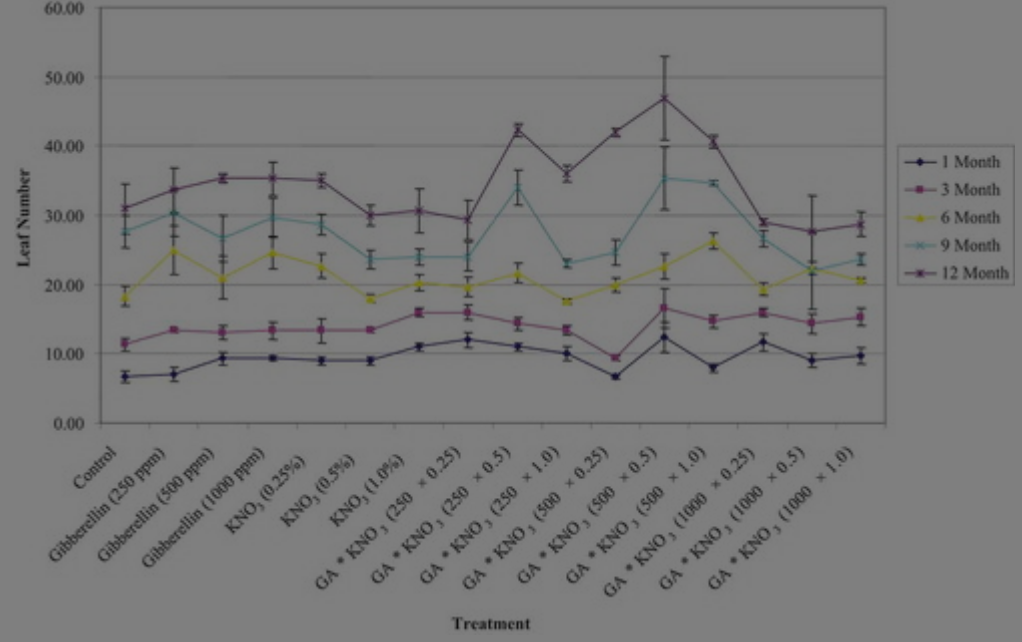
The single factors (GA_3 and KNO_3 concentration) significantly influenced the leaf area ($p \leq 0.05$) (Table 3). Seeds treated with the different concentrations of GA_3 are found to have more leaf area as compare to KNO_3 treated seed and untreated seeds (control). A lower concentration of GA_3 (250 mg L^{-1}) had a favorable effect on leaf expansion. While at a higher concentration of GA_3 treatment leaf area found to be non-significantly decreased. Similar to GA_3, leaf area of treated KNO_3 seeds was declined with increases of KNO_3 concentration. The GA_3 and KNO_3 treatments significantly affected the leaf area. The maximum leaf area was recorded in the plants treated with GA_3 250 × KNO_3 1%, followed by GA_3 500 × KNO_3 0.5%. While, treatment combination, GA_3 1000 mg L^{-1} × KNO_3 0.25% had some adverse effect on leaf expansion, and it resulted in minimum leaf area. Similar, leaf expansion at lower GA_3 concentration (250 mg L-1) was observed in Eriobotrya japonica (Shabaq, 2013), Citrus limon (Dzayi and Rahman, 2010) and Citrus aurantifolia (Meshram et al., 2015).

In general, numbers of leaves per plant were not improved by the pretreatment of GA_3 and KNO_3 (Figure 3). However, numbers of leaves per plant were significantly improved with combined application of GA_3 and KNO_3. The presowing treatment of seeds with GA_3 500 mg L^{-1} × KNO3 0.5%, led to maximum increase in the leaf number per plant, followed by seeds treated with GA_3 250 mg L^{-1} × KNO_3 0.5%. In contrast, GA_3 seeds soaked in higher gibberellin concentration (1000 mg L^{-1}) supplemented with medium or high KNO_3 (0.5% or 1%) concentration for 24 h, gave the minimum increase in the leaf numbers per plant. The various previous study reported the opposite relations between leaf growth and plant height increment due to GA_3 treatment (Arney and Mancinelli, 1966). These trends are concurrent with the present study. GA_3 pregerm a papaya (Parab e y due to the stimulat 2017).

Figure 3 GA_3 (in ppm) and KN



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Leaf Fresh and Dry Weight

The result indicated that the highest leaf fresh and dry weight was achieved when the C. sinensis seeds were treated with the aqueous solution of GA_3 250 mg L^{-1}, supplemented with the KNO_3 1%, followed by the solution containing GA_3 500 mg L^{-1} and KNO_3 0.25%. The minimum leaf fresh weight was noted under the highest KNO_3 (1% conc.) treatment. In general, fresh weight of leaf was higher when seeds were treated with GA_3 than a KNO_3 solution; further fresh weight was decreased when GA_3 concentration increased from 0.25% to 1%. While the lowest dry weight was found under maximum concentration of GA_3 treatment and KNO_3. It was observed that higher concentration of both the chemicals (GA_3 and KNO_3) resulted in the decline in the dry weight c…lant weight, specific …y, higher fresh an…s of Carica papaya …rsha et al., 2017). M… GA_3 (250 mg L^{-1})… …er shoot and … and Rahman… …h and dry shoot we… …mobilization of water… …h ultimately leads to …arsha et al.,

2017). While, higher mobilization of photosynthetic products was also taken place in the KNO_3 treated seeds in the early stage of seed germination (Lay et al., 2015).

Leaf Nutrient Traits

The different concentration of GA_3 × KNO_3 affects the leaf N, P, K, and Na content significantly (Table 3). The leaf N content was lower under the different single GA_3 and KNO_3 treatments as compared to control. However, combination treatment of GA_3 1000 mg L^{-1} and KNO_3 0.25% resulted in highest leaf N content. In general leaf N content was higher in the plants treated with GA_3 as compare to KNO_3 treatments. The leaf P content was higher under the GA_3 treated plants it was highest under GA_3 250 mg L^{-1} treatment closely followed by GA_3 500 mg L^{-1} × KNO_3 1% treatment. The combined application of GA_3 1000 mg L^{-1} × 0.5% KNO_3 resulted minimum leaf P content. In general higher leaf P content was achieved when seeds were treated with GA_3 for 24 h than KNO_3 treatment for the same duration. Percentage leaf K content was lower for GA_3 and KNO_3 treated seeds as compared to seeds treated with distilled water (control). However, among the treatments, the highest K content was noted when seeds were soaked in GA_3 500 mg L^{-1} followed by KNO_3 (0.25%). The lowest leaf K content was reported when the seeds were soaked in the aqueous solution of GA_3 1000 mg L^{-1} supplemented with KNO_3 1% concentration. The treatment of GA_3 resulted significantly high leaf Na content than control. However, there was no significant difference between seeds soaked in H_2O (control) and KNO_3 solution for leaf Na content. The seeds soaked in GA_3 (500 mg L^{-1}) and KNO_3 0.25% had the highest leaf Na content. Whereas, lowest leaf Na content was recorded when seeds were treated with an a 0.25% for 24 h.

Increase GA_3 and KNO_3 wa gher leaf nitrogen ent GA_3 appli mbination of GA_3 (1 emi (2014) noted th the applicati opersicum leaves. T milar effect was obs on of

were responsible for increase in leaf NPK contents (Marschner, 2012). Leaf sodium content was found to be decreased with combined application of gibberellins and potassium nitrate in the study. The similar antagonistic correlation was also observed by Song and Fujiyama (1996).

Thus, the external application of KNO_3 and GA_3 showed a synergistic effect in promoting seed germination and seedling vigor of Cordia sinensis seed in the present investigation. In conclusion, it can be stated that the application of GA_3 (250 ppm) and KNO_3 (1%) is a simple, effective, and practical method for improving seed germination and seedling growth of Cordia sinensis. Although, further work might be useful for in-depth understanding on effects of chemicals in Cordia sinensis.

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Disclosure Statement

The authors declare that they have no conflict of interest.

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