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Biological activity of some fruit leaves and chemical investigation on *Dillenia indica* leaves

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Abstract: Plant leaves contain various kinds of chemical substances which are mostly beneficial and environmentally friendly. A study was conducted on activities of aqueous extracts of leaves of ten fruit plant species on four summer vegetable crops. The experiment was conducted to study about the naturally occurring growth substances in aqueous extracts of ten fruit plant leaves viz., mango (*Mengifera indica*), black berry (*Syzygium cumini*), jack fruit (*Artocarpus heterophyllus*), litchi (*Litchi chinensis*), wood apple (*Aegle marmelos*), Indian dillenia (*Dillenia indica*), papaya (*Carica papaya*), banana (*Musa sp.*), guava (*Psidium guajava*), and olive (*Elaeocarpus floribundus*) and the vegetables were country bean (*Lablab niger*), yard long bean (*Vigna radiata*), okra (*Hibiscus esculentus*) and swamp cabbage (*Ipomea aquatica*) that were used for germination and growth studies. An attempt was also for studying the chemical properties (compound and structure) on the best performed fruit leaves extract that was extracted by chloroform extract. The aqueous extracts of indian dillenia significantly increased germination of country bean, yard long bean, okra and swamp cabbage seeds in comparison with control. The germination percentage were 100%, 99%, 73% and 60% in country bean, yard long bean, swamp cabbage and okra seeds, respectively, treated with indian dillenia compared with control. The effect of aqueous extract of indian dillenia also increased growth of radical and plumule length of country bean, yard long bean, okra and swamp cabbage seedlings. The thin layer chromatography (TLC) of chloroform extracts of indian dillenia indicated four distinct compounds at Hexane: Ethylacetate (9:1, v/v,). Crude compounds were purified and isolated by column chromatography. H-NMR and IR study indicated that the fractions might have steroid and triterpinoid type of compounds.
Key words: Aqueous extracts, Chemical investigation, Growth regulator, Germination, Indian dillenia

Introduction

There are different types of plants such as, fruit trees, herbal plants, narcotic crops, flower plants, weeds, etc. Different types of naturally occurring organic and bioorganic compounds have been isolated from them. Most of them have effective medicinal, pesticidal or toxic and/or growth regulatory values which are safer than synthetic one. Plants are the richest source of renewable bio-active organic chemicals.

The plants are a vast reservour of compounds with a wide range of biological activities. There are about 2000 plant species reported to possess pest control properties (Ahmed *et al.* 1984). The plant product includes oil, extracts, dried leaves, fruits, seeds, rhizomes etc. The strongest inhibitory effect of aqueous extracts from *Eupatarium adenophorum* on wheat seed germination, radical and plumule growth were reported by Tripathi *et al*. (1981). The extracts of Leaves, stems and radicals of lantana plant have the inhibitory effects growth of some plant species (Wadhwani and Bhardwaja 1981). Tripathi *et al.* (1981) reported that the strongest inhibitory effect of aqueous extracts of *Eupatorium adenophorum* on wheat seed germination, radical and plumule growth. Bhuyan and Deka (1999) reported that increased germination of *Phaius tankervillae* in nitch and nitsch medium and germination could be further enhanced to 99% by supplementing the medium with coconut water or pineapple juice. Rice (1974) reported that secondary compounds are either bi-products of metabolism or waste products stored in the vacuoles to prevent deleterious effects on the producing plant. Compound implicated as likely phytotoxic agents are phenolic acids, terpenoids, flavonoids, alkaloids, cynogenicglycosides, quinines and amino acid derivatives. Several investigators have reported that the effect of extracted primary and secondary metabolites from different weeds on germination, growth and development of various crops and some have insecticidal effects (Kohata *et al.* 2004). The plant synthesis different types of hormones like auxin, soprene, abcsisic acid, ethylene and terpene compounds used as defense mechanism and growth substances. Above information reported on germination, inhibitory or increasing influence, insecticidal activity as well as nutrient assimilation and other important biological activity of aqueous extracts of plant/weeds leaves residues during germination of different crop/weed. There is very limited works regarding the biologically active compounds in common fruit leaves in Bangladesh. The growth inhibitory or enhancing effect of different plant extract on germination of different crops plants are very important in agriculture but it is essential to investigate the chemical ingredient that are responsible for the growth regulating properties. Therefore, an investigation has been carried out using some common fruit leaves to investigate the growth regulatory effect of aqueous extract of different fruit leaves and to isolate the biologically active compounds from effective extracts.

Materials and Methods

The experiment was conducted at the Laboratory of Agricultural Chemistry and Biochemistry in Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh during the period of January 2007 to June 2007 to study the effect of naturally produced growth substances in ten fruit plant leaves viz., mango (T_2), black berry (T_3), jack fruit (T_4), litchi (T_5), wood apple (T_6), indian dillenia (T_7), papaya (T_8), banana (T_9), guava (T_{10}), olive (T_{11}) where tap water (T_1) was used for control. The aqueous extract was treated separately on the following vegetable crops such as country bean, yard long bean, okra and swamp cabbage along with the attempt for chemical investigation on the effective leave extract.

Preparation of Aqueous Leaf Extracts: Exactly 100 g of each fresh leaf was taken separately for each tested plant and chopped followed by making paste using blender machine with required amount of water. The paste was transferred to a 500 ml reagent bottle and the 400 ml water was added to it and placed for 72 hours at room temperature (25±2 °C). The reagent bottles were stirred with glass rod at regular interval. After 72 hours the aqueous slurry was filtered through Whatman filter paper

No.1 and stored in glass bottles in a refrigerator. The filtrates of individual leaf extracts were stored and used in the experiment.

Growth of Vegetable Crops: petridish experiments was done to observe the germination percentage, radical and plumule growth for country bean, yard long bean, okra and swamp cabbage seeds in the Laboratory room. For this a 10 cm diameter clean petridish was used in which with two round sheets of 10 cm diameter filter paper was placed. Then 15 ml of each aqueous extracts was separately added in each petridish. Then twenty five (25) seeds of each vegetable crop were placed on filter paper that was previously placed on each petridish. Each treatment were replicated into three (3) times. All the petridishes were kept in natural diffused light under laboratory conditions at the temperature of 26±2 °C. To moisten the filter paper, 5 ml water was added if the filter paper were dried (Dubey 1973). 15 ml distilled water was added for control instead of aqueous extract. Germination rate was monitored regularly and after germination of seeds growth of plumule and radical were recorded everyday and continued until germination was completed. For this, length of radical and plumule have been recorded for seven days. The collected data were analyzed statistically and the differences between means were compared by using Duncan's New Multiple Range Test (DMRT).

Isolation of Crude Compounds Using Chloroform: For isolation of crude compounds, 100 g powder of leaf was taken in 2.5 L reagent bottle and 250 ml chloroform was added to the powder and it was kept 72 h with regular interval of shaking. Then it was filtered by using Whatman filter paper No.1. The extract was collected in 500 ml reagent bottle. More 200 ml of chloroform was added to the residue again, the reagent bottle was again kept for next 72 h with shaking at regular interval followed by complete filtering with Whatman filter paper No.1. The collected both chloroform extracts were mixed together. The solvent was evaporated from the extract by using rotary film evaporator under reduced pressure. After evaporation the semisolid crude was stored in refrigerator at 4 ^{o}C for chemical investigation. TLC was prepared for the Indian dillenia as the most potential and compounds were detected by using the solvent hexane and ethylalcohol in the ratio of 9:1 (v/v).

Infrared (IR) spectroscopy study: IR (Infrared Spectroscopy) was measured using crude with IR spectrometer (Model: FTIR-8900 SCHIMAZU, Japan) from BCSIR (Bangladesh Council of Scientific and Industrial Research) Dhaka, Bangladesh.

^{1}H-NMR (Nuclear Magnetic Resonance) study: ^{1}H-NMR of spectra was measured with NMR spectrometer (Model: 400MHZ, Switzerland) from BCSIR (Bangladesh Council of Scientific and Industrial Research) Dhaka, Bangladesh.

Results and Discussion

Biological Activity (Germination and Growth): The country bean, swamp cabbage, yard long bean and okra showed a wide variation in germination and radical and plumule growth using the aqueous extracts of different fruit leaves tested. The aqueous extracts of indian dillenia (T_7) significantly increased the germination of country bean seeds compare to the control. The highest germination rate was found in country bean treated with the extracts of indian dillenia leaves but the lowest (49%) recorded with the extracts of wood apple leaf extracts. The second highest germination rate was 96% in seeds treated with olive leaves followed by black berry (84%), jack fruit, T_4 (76%), litchi, T_5 (75%) and papaya, T_8 (72%) which was statistically similar. The effect of aqueous extracts of different fruit leaf on the radical length of country bean seedlings were significantly increased by the aqueous extracts of indian dillenia compared with control. The highest radical length of country bean seedling (5.10 cm) was recorded in seeds treated with indian dillenia. The second highest radical length 3.70 cm which was obtained in seeds treated with litchi olive (3.397 cm), guava (3.34 cm) and mango (3.32 cm) which was statistically similar. The third highest radical length (T_8) 2.96 cm was found in seeds treated with papaya followed wood apple (2.81 cm), Black berry 2.55 cm, control 2.09 cm and Litchi 1.58 cm which were statistically identical. The lowest radical length of country bean seedling (1.23 cm) was obtained in seed treated with banana. The plumule length of country bean seedling 7.41cm was recorded in seeds treated with indian dillenia and 6.40 cm was treated with distilled water or control and followed by 5.92 cm in seeds treated with Jack fruit 4.88 cm, Papaya 4.52 cm, Wood Apple 4.36 cm, Mango 4.27 cm, Guava 4.17 cm, Olive 3.75 cm, Banana 3.17 cm which was statistically similar. The lowest plumule length of country bean seedling (2.71 cm) was recorded in the seed treated with litchi. The highest germination and radical and plumule length of country bean seedling was found in indian dillenia aqueous extract possibly due to some growth regulator or bioactive substances present in these species. Similar results were also reported in Kocacaliskani and Terzii (2001) about the growth increasing effect of leaf extract of walnut on seed germination and seedling growth of muskmelon. The highest germination percentage (73%) was found in seeds treated with indian dillenia (T_7) over the control (67%) which was statistically identical. On the contrary, the lowest germination percentage (54%) was found for the seeds treated with wood apple (T_6). The second highest germination percentage (72%) was obtained from the seeds treated with T_3 (63%), T_2 (62%), T_8 (58%) and T_{11} (56%) which were statistically similar. Table 2 shows that radical length markedly increased in swamp cabbage seedlings (2.85 cm) treating with indian dillenia (T_7) over the control. The second highest radical length of swamp cabbage seedling (2.57 cm) was found in control (T_0) followed by T_8 (2.43 cm), T_5 (2.39 cm) and T_2 (1.92 cm) which were statistically identical. Similarly the radical length of T_2 (1.92 cm), T_3 (1.66 cm) and the effect of swamp cabbage seedling was recorded statistically similar. The highest length of plumule was 2.92 and 2.58 cm was for aqueous extract of T_7. The lowest plumule length (0.89 cm) was for swamp cabbage seedlings treated with wood apple (T_6) and second highest plumule length (2.32 cm) was recorded in seeds treated with litchi (T_4) extract. The plumule length of swamp cabbage seedlings reduced in

seed treated with (T_{11}) (1.91 cm), (T_2 (1.59 cm), and (T_8) (1.45 cm) statistically identical. The increasing tendency of plumule length in aqueous extract treated seedlings might be due to the presence of some regulatory compounds or other bioactive compounds.

Table 1. Effect of fruit leaf extracts on germination and primary growth of country Bean

Treatments	Germination (%)	Radical length (cm)	Plumule length (cm)
Water (control)	8 2.67 bc	2.09 bcd	6.40 ab
Mango	81.33 c	3.32 abc	4.36 ab
Black berry	84.00 bc	2.55 bcd	3.17 ab
Jack fruit	76.00 c	3.70 ab	5.92 ab
Litchi	74.67 c	1.58 cd	2.71 b
Wood apple	48.67 d	2.81 bcd	4.52 ab
Indian dillenia	100.0 a	5.10 a	7.41 a
Papaya	71.67 c	2.96 bcd	4.88 ab
Banana	80.00 c	1.23 d	3.75 ab
Guava	78.67 c	3.34 abc	4.25 ab
Olive	96.00 ab	3.39 abc	4.17 ab
Sd	3.384	0.4231	1.013

Table 2. Effect of fruit leaf extracts on germination and primary growth of swamp cabbage

Treatments	Germination (%)	Radical length (cm)	Plumule length (cm)
Water (control)	66.67 ab	2.57 ab	2.58 ab
Mango	61.33 ab	1.92 bc	1.59 bc
Black berry	63.00 ab	1.66 c	2.32 ab
Jack fruit	48.00 b	1.63 c	2.03 abc
Litchi	53.33 ab	2.39 ab	2.12 abc
Wood apple	5.33 c	1.53 c	0.89 c
Indian dillenia	72.67 a	2.85 a	2.92 a
Papaya	58.67 ab	2.43 ab	1.45 bc
Banana	46.67 b	1.60 c	2.29 ab
Guava	72.00 a	1.77 c	1.91 abc
Olive	56.00 ab	1.28 c	2.07 abc
Sd	6.15	0.16	0.28

Table 3. Effect of Fruit Leaf extracts on germination and primary growth of Yard long bean

Treatments	Germination (%)	Radical length (cm)	Plumule length (cm)
Water (control)	96.00 a	2.480 b	5.36 b
Mango	69.33 b	2.94 b	3.49 bcd
Black berry	36.33 d	1.85 b	2.18 cd
Jack fruit	66.67 bc	1.87 b	3.44 bcd
Litchi	64.00 bc	2.08 b	2.05 d
Wood apple	42.00 cd	2.24 b	3.14 cd
Indian dillenia	98.67 a	5.53 a	7.90 a
Papaya	86.67 ab	1.37 b	2.49 cd
Banana	85.33 ab	1.46 b	2.04 d
Guava	68.00 b	1.89 b	3.41 bcd
Olive	68.00 ab	3.04 b	4.20 bc
Sd	6.048	0.5910	0.4615

During the effect of aqueous extracts of leaves on yard long bean seed germination. The highest germination percentage (98.67%) was recorded in seeds treated with indian dillenia (T_7) and 96% was founded in seeds treated with control was statistically identical over the control and other treatments germination percentage (86%) was founded in seeds treated with Banana (T_8), (86%) (T_{11}) and (85%) (T_9) was statistically similar. The second superior seed germination percentage on yard long bean seeds 69% was found in seeds treated with Mango (T_2), 68% Guava (T_{10}), 66% Jack fruit (T_6), 64% litchi (T_5) which were statistically similar among themselves and the lowest germination percentage36% seeds treated with Black berry (T_3).

The highest radical length of yard long bean (5.53 cm) was found in seeds treated with indian dillenia (T_7), and the seeds treated with (T_{11}) (3.04 cm), (T_2) (2.94 cm), (T_1) (2.4 cm), (T_6) (2.24 cm), (T_5) (2.08 cm), (T_{10}) (1.89 cm),

(T_4) (1.87 cm), (T_3) (1.85 cm), (T_9) (1.46 cm) and (T_{11}) (1.37 cm) which were statistically similar. The highest plumule length of yard long bean seedlings (7.9 cm) were founded in seeds treated with Indian dillenia (T_7) and second highest plumule length of yard long bean seedlings (5.36 cm) in seeds treated with control. The plumule length of yard long bean (T_{11}) (4.2 cm), (T_2) (3.49 cm), (T_4) (3.44 cm), and (T_{10}) (3.41 cm) were statistically identical. The seeds treated with the treatment of (T_6) (3.14 cm), (T_8) (2.49 cm), (T_3) (2.18 cm) and (T_5) (2.05 cm) which were statistically similar. The lowest plumule length of yard long bean seedling 2.04 cm seed treated with Banana. The increasing tendency of germination rate and radical-plumule length treated with the aqueous extract of indian dillenia might be due to the presence of some chemicals (Table 3).

It was clear that the highest germination percentage (60%) was found in seeds treated Indian dillenia (T_7) over the control. Where as the lowest germination percentage (2.333%) was found in okra seeds treated with Wood apple (T_6). The other germination percentage (40.00%) was obtained from in seeds treated with T_2, T_1 (38%), T_{10} (31.67%), T_4 (29%), T_5 (28%), T_{11} (27%), T_9 (25%) and T_3 (24%), which were statistically similar. It is no doubt to say that the highest seedling radical length of okra (3.43 cm) was found in seeds treated with indian dillenia (T_7). Seeds treated with Banana (T_8) (2.43 cm), 2.40 cm was recorded in seeds treated with control (T_1), (T_5) (2.39 cm), (T_2) (2.24 cm), (T_3) (1.66 cm) were statistically identical. Radical length (1.42 cm) of okra seedlings were recorded in seeds treated with Olive and (T_4) (1.42 cm), (T_{11}) (1.34 cm) which was statistically same. The highest plumule length of okra seedlings (3.33 cm) were founded in seeds treated with indian dillenia (T_7) and lowest plumule length of okra seedlings (0.77 cm) in seeds treated with Wood apple (T_3) (Table 4). The other plumule length of okra seedlings (2.67 cm) were recorded in seeds treated with olive and (T_1; 2.62 cm), (T_5; 30 cm) statistically similar. The another plumule length of okra seedlings (2.30 cm) were recorded in seeds treated with litchi (T_5), 1.99 cm in seeds treated with banana (T_9) and Jack fruit (T_4; 1.91 cm) statistically similar. The highest seed germination percentage and radical-plumule length was obtained in those seeds treated with indian dillenia due to presence of some chemicals or growth regulator(s). Similar findings was also by Bhyan and Deka (1999) that better germination of *Phaius tankervillae* in Nitsch and Nitsch medium could be further enhanced to 99% by supplementing the medium with coconut water or pineapple juice (20 ml L^{-1}).

Table 4. Effect of fruit leaf extracts on germination and primary growth of okra

Treatments	Germination (%)	Radical length (cm)	Plumule length (cm)
Water (Control)	37.33 b	2.40 c	2.62 ab
Mango	40.00 b	2.24 bc	1.57 bc
Black berry	23.33 b	1.66 bcd	1.69 bc
Jack fruit	28.33 b	1.42 d	1.91 bc
Litchi	23.33 b	2.39 bc	2.30 ab
Wood apple	2.333 c	1.10 d	0.77 c
Indian dillenia	60.00 a	3.43 a	3.37 a
Papaya	28.33 b	2.43 b	1.33 bc
Banana	25.00 b	1.60 cd	1.99 bc
Guava	31.67 b	1.42 d	1.83 bc
Olive	27.33 b	1.34 d	2.67 ab
Sd	4.956	0.1862	0.2955

Chemical Investigation of the Effective Fruit Leaves: The results in this experiment indicates that the aqueous extracts of different plant species have inhibitory or increasing activity on germination and growth on radical and plumule length or early growth of vegetable crop such as country bean, yard long bean, okra, swamp cabbage. The interesting decreasing tendency as well as growth promoting activity on germination rate, shoot and root length growth by Indian dillenia on country bean, swamp cabbage, yard long bean and okra seeds, showed potentially bioactive. Considering this, we purified the crude of ethanol extract of Indian dillenia and four distinct compounds were detected by TLC at Hexane: Ethylacetate (9:1 v/v) as Cha_1, Cha_2, Cha_3, and Cha_4, respectively, followed by purification using column chromatography.

It showed the intensity of non-polar compound like Cha_1 was too much high in comparison with others. These compounds are detected in iodine tank and the R_f value was calculated. Table 5 shows the higher R_f value indicated as most non polar compound and the least R_f value indicated as most polar compounds.

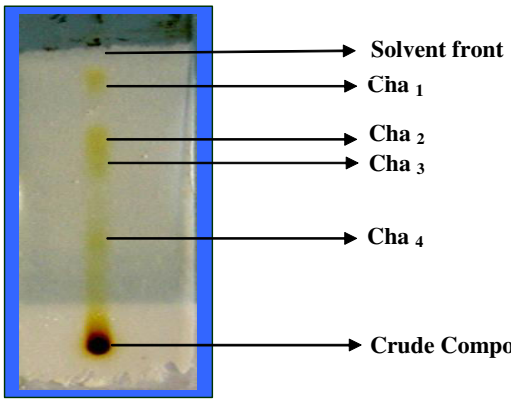

Fig. 1. TLC of chloroform crude extract of indian dillenia using the solvent hexane and ethylalcohol in the ratio of 9:1 (v/v)

Table 5. The R_f value of crude extract of indian dillenia

Name of plant species	Hexane:Ethylacetate	Detected component	R_f value
Indian dillenia	9:1	Cha_1	0.83
		Cha_2	0.67
		Cha_3	0.53
		Cha_4	0.18
Indian dillenia	8:1	Cha_1	0.95
		Cha_2	0.75
		Cha_3	0.55
		Cha_4	0.33
Indian dillenia	6:1	Cha_1	0.98
		Cha_2	0.83
		Cha_3	0.75
		Cha_4	0.53

Purification of chloroform crude extract of indian dillenia: The components present in crude extract of indian dillenia was isolated using column chromatography eluting with hexane: ethylacetate (40:1 and 30:1, v/v) and were collected as fractions designated as Cha_1 (3-6), Cha_2 (8-14) and Cha_3 (20-30). TLC examination of the above fractions indicated single spot and these samples were collected in different round bottom flask the solvent was evaporated under reduced pressure, and isolated components was stored separately in the refrigerator for investigation.

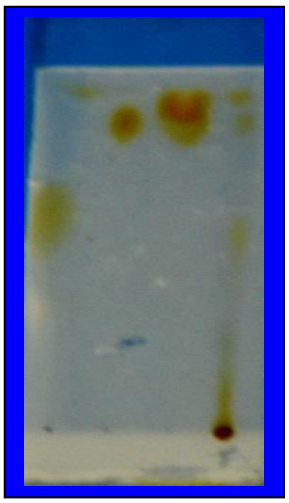

Fig 2. Comparative TLC of crude compound and purified compound after column chromatography (Hexane: Ethylacetate = 9:1, v/v)

Spectral study of purified compounds: The purified components were studied by ^{1}H-NMR and IR spectroscopy and indicated that

^{1}H-NMR of Cha_1 (400MH_3, CD Cl_3): δ= 5.30 (m), 5.08 (t), 4.56 (t), 2.28 (t), 1.99 (m), 1.88 (m), 1.74 (m), 1.67 (s), 1.59 (t), 1.24 (m), 1.04-1.00 (m), 0.95 (s), 0.88-0.83 (m), 0.66 (m), 0.56 (m), 0.33 (m).

^{1}H-NMR of Cha_2 (400 MH_3, CD Cl_3) showed δ = 5.34 (t), 5.04 (t), 4.55 (m), 3.62 (S), 2.79-2.75 (dd), 2.28 (t), 2.04-2.03 (m), 1.88 (m), 1.74 (s), 1.67 (m), 1.61-1.59 (m), 1.29-1.24 (m), 1.12-1.08 (m), 1.02-1.01 (m), 0.88-0.77 (m), 0.56 (dd), 0.33-0.32(d).

^{1}H-NMR of Cha_4 (400 MH_3, CD Cl_3) showed δ = 5.33 (m), 2.33 (m), 1.98 (m), 1.82 (m), 1.60 (m), 1.48 (m), 1.24 (m), 1.00 (S),

IR (Infrared Spectroscopy) of Cha_1 (CD Cl_3 solution): Cha_1 υ_{max} = 2858.3 cm^{-1} (br), 1728.1 (s), 1579.6 (s), 1579.6 (s), 1463.9 (s),1379 (s), 1272.9 (br), 1122.5 (s,) 1122.5 (s), 1072.3 (s), 1039.6 (s)

IR (Infrared Spectroscopy) of Cha_2 (CD Cl_3 solution): Cha_1 υ_{max} = 2856.4 cm^{-1} (br), 1726.2 (br), 1579.6 (s), 1463.9(s), 13.79(s), 1272.9(br), 1122.5(br), 1072.3(s), 1039.6(s), 983.6 (br).

IR (Infrared Spectroscopy) of Cha_4 (CD Cl_3 solution): Cha_1 υ_{max} = 2860.2 cm^{-1} (br), 1726.2 (br), 1579.6 (br), 1463.9 (br), 1380.9(s), 1274.9 (br), 1122.5 (br), 1072.3 (s), 1039.6 (s), 958.6 (s).

Attempt for determining the structure of purified compounds: From the above IR and ^{1}H- NMR for Cha_1, Cha_2 and Cha_4, it is observed that Cha_1 showed one absorption at 1728.1 cm^{-1} indicating the presence of carbonyl group. So, Cha_1 might have simple mono or sesquiterpenoid with carbonyl group. Cha_2 showed one absorption at 1726.2 cm^{-1} and 1579.6 cm^{-1} and ^{1}H-NMR indicated that δ 0 to 2.8 ppm region is same as the sample Cha_1. Therefore, sample Cha_2 might contain Cha_1 with one or more compounds with olefinic group. From IR spectra it is clear that it is a simple ketone with also some impurities of Cha_1 and Cha_2 and alcoholic compound. From the comparative study, it is evidently shows that cha_1 is almost pure compound in comparison to other isolated compounds. Mass spectra of Cha_1 are in progress, after receiving mass spectra of the structure of Cha_1 will be designed, which will be reported in due course.

Considering the biological activities of aqueous extract of tested plant species, indian dillenia enhanced germination rate, radical and plumule growth of country bean, swamp cabbage, yard long bean and okra. The most effective extract was the indian dillenia among the tested extracts. Therefore, compounds responsible for growth promoting property have been isolated and purified. Chloroform extracts of indian dillenia might contain mono or sesquiterpenoid. The present findings also infer that it deserves further field experiment using the indian dillenia extract as growth promoting substance. Leaves of this plant need further chemical investigation with polar solvent for studying detail chemical properties.

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