# Antibacterial, anti-inflammatory and anti-oxidatant activities of various isolated compounds from *Cratoxylum* species

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**Abstract**: The objective of this study was to investigate the bioactivity of twenty-nine known isolated compounds from *Cratoxylum* species including three anthraquinones, four triterpenes, and twenty-two xanthones. All isolated compounds were subjected to antibacterial, anti-inflammatory and anti-oxidant activities. Cytotoxicity evaluations were performed by MTT assay. The anti-oxidatant activity was performed using DPPH assay. The anti-inflammatory activity was evaluated from the production of cytokines TNF-α and IL1-β using ELISA assay. Human gingival fibroblasts and monocytes could tolerate both anthraquinones and triterpenes. All isolated anthraquinones showed moderate-to-high antibacterial efficacy while compound A3 also demonstrated moderate anti-inflammatory effect. None of the isolated triterpenes, except for T1, inhibited the expression of TNF-α. A number of isolated xanthones was toxic to HGFs and monocytes. Compound X5, X14 and a 1:1 mixture of X5 and X6 showed comparative anti-inflammatory activity to dexamethasone. Several triterpene and xanthone compounds also expressed antibacterial effect against *P. gingivalis*. Some isolated xanthones exerted anti-oxidant activity comparable to ascorbic acid. Accordingly, selected pure compounds from plants of *Cratoxylum* genus might be of benefit in developing medications that are important in treating periodontal diseases.

**Keywords**: *Cratoxylum*, anthraquinones, triterpenes, xanthones, antibacterial, anti-inflammatory, anti-oxidant.

## INTRODUCTION

Periodontal disease is a chronic inflammatory reaction of the periodontium to injury or to infectious or toxic stimulation from specific bacteria. The irritation and tissue injury is typically limited by the function of leukocytes, which kill the bacteria and remove dead cells. Moreover, pro-inflammatory cytokines or inflammatory mediators (e.g. reactive free radicals) secreted from immune-modulatory cells seems to prolong existing inflammation which continue to cause or prolong a variety of pathological conditions. Accordingly, a treatment modality using mechanical debridement focusing on the removal of bacterial accumulation alone may not be effective enough to halt disease progression. Even though the use of chemotherapeutics has been effectively used as an adjunct to treat this chronic disease, the question of their side effects and especially drug resistance concerns (Köhler *et al*., 1999) has urged dental researchers to search for alternatives. Accordingly, attention has been drawn to the use of plant materials and isolated compounds from medicinal plants.

*Cratoxylum formosum* ssp. *pruniflorum* known in Thai as "Tiu Khon" and *Cratoxylum cochinchinese* (*Lour*.) Blume (Tiu Kliang) are shrubs or small trees, which can reach up to 20 and 33 meters tall, respectively. They belong to the family *Clusiaceae* (*Guttiferae*), which can be found in several Southeast Asian countries. Tiu Khon has been traditionally known for its tonic, stomachic, and diuretic effects whereas Tiu Kliang has yet shown a particular pharmacological potential (Grosvenor *et al*., 1995). Bioactive activities, including antimicrobial, anti-inflammatory and anti-oxidant, of the crude extracts and pure isolated compounds from this plant family have been reported elsewhere (Kuvatanasuchati *et al*., 2011; Sobral *et al*., 2009; Tsaffack *et al*., 2009; Banerjee *et al*., 2000). Nevertheless, there is scarce evidence indicating which secondary metabolites within these medicinal plants are responsible for their bioactivities. Thus, the objective of this study is to investigate these plants from phytochemical effectiveness of various isolated compounds from *Cratoxylum* species by evaluating antibacterial, anti-inflammatory, and anti-oxidant activities.

## MATERIALS AND METHODS

### *Cell lines and chemicals*

The monocyte cell line U937 was kindly provided by Siriraj Medical School. HGF cell line (ATCC CRL-2014) was purchased from ATCC (USA). Growth media (RPMI 1640 containing 2mM L-glutamine and Dulbecco's

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Modified Eagle's Medium (DMEM)) and antibiotic-antimycotic solution were purchased from Gibco (Grand Island, NY, USA). Fetal bovine serum (FBS), Tryphan blue, 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide (MTT), cell culture grade dimethylsulphoxide (DMSO), LPS (*Esterichia coli* 026:B6), and phorbol-12-myristate-13-acetate (PMA), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Co. (St. Louise, MO, USA). Dexamethasone (DM) was purchased from APP Pharmaceuticals, LLC (Schaumberg, IL, USA). ELISA kits were purchased from Thermo Scientific (Rockford, IL, USA).

***Plant materials***

The dried whole plants of *Cratoxylum formosum* ssp. *pruniflorum* were collected in northeastern part of Thailand in May 2004 and were identified by Prof. Puangpen Sirirugsa from Prince of Songkla University. A voucher specimen (No. 0012677) has been deposited at the Prince of Songkla University Herbarium. The green fruits of *Cratoxylum cochinchinense* were collected in southern part of Thailand in October 2007. The plant material was also deposited in Prince of Songkla University Herbarium (No. SL-1).

***Isolation and extraction***

The powdered dried barks of *C. formosum* ssp. *pruniflorum* (4.0kg) were extracted two times with $CH_2Cl_2$ (2×20L) by maceration at room temperature and were further evaporated under reduced pressure to obtain a crude $CH_2Cl_2$ extract (76.28g). The crude $CH_2Cl_2$ extract was fractionated on a silica gel QCC by using a step gradient of EtOAc and acetone in *n*-hexane as an eluent to give 10 fractions (A1-A10). Fraction A1 (2.01g) was chromatographed on a silica gel (CC) column with acetone-*n*-hexane (5:95) as an eluent to give 3 sub fractions (A1A-A1C). Subfraction A1B was separated on a silica gel column eluting with EtOAc-*n*-hexane (10:90) to give vismiaquinone A (A1, 11.3mg) (Goncalves and Mors, 1981) and madagascin (A2, 15.6mg) (Ritchie and Taylor, 1964). Fraction A2 (58.06g) was purified on a silica gel column by using a step gradient of EtOAc in *n*-hexane to give 8 subfractions (A2A-A2H) and macluraxanthone (X11, 150.0mg) (Delle Monache *et al*., 1981). Sub fraction A2C (120.02g) was chromatographed on a silica gel (CC) column with EtOAc-*n*-hexane (20:80) to give 3-geranyloxy-6-methyl-1,8-dihydroxyanthraquinone (A3, 68.2mg) (Botta *et al*., 1983). Fraction A3 was fractioned on a silica gel column eluting with acetone-*n*-hexane (10:90) to afford 5 fractions (A3A-A3E). Subfraction A3D was separated on a silica gel column using acetone-*n*-hexane (15:85) to give gerontoxanthone I (X9, 25.0mg) (Chang *et al*., 1989).

The dried roots of *C. formosum* ssp. *pruniflorum* (5.0kg) were extracted two times with $CH_2Cl_2$ (2×20L) by maceration at room temperature. The volume of combined $CH_2Cl_2$ extracts were reduced under vacuum to give a crude $CH_2Cl_2$ extract (58.87g), which was chromatographed on a silica gel QCC with a step gradient of acetone in *n*-hexane to obtain 8 fractions (B1-B8). Fraction B2 was chromatographed over a silica gel column eluted with $CH_2Cl_2$-*n*-hexane (20:80) to afford 4 subfractions (B2A-B2D). Subfraction B2A was separated on a silica gel with EtOAc-*n*-hexane (30:70) to give $\beta$-mangostin (X15, 45.0mg). Subfraction B2B was further purified on a silica gel with acetone-*n*-hexane (10:90) to give $\alpha$-mangostin (X14, 15.0mg) (Mahabusarakam *et al*., 1987). Fraction B3 (2.56g) was chromatographed on a silica gel (CC) column with acetone-*n*-hexane (15:85) to afford dulxisxanthone F (X13, 10.2mg) (Deachathai *et al*., 2006). Fraction B6 was fractioned on a silica gel QCC eluting with a step gradient of acetone in *n*-hexane to afford 10 subfractions (B6A-B6J). Subfraction B6H was fractioned on a silica gel QCC eluting with a step gradient of acetone in *n*-hexane to afford 11 subfractions (B6H1-B6H11), 1,3,7-trihydroxy-2,4-diisoprenylxanthone (X4, 150.2 mg) (Nguyen and Harrison, 1998), cochinchinone A (X5, 80.7 mg) (Mahabusarakam *et al*., 2006) and pruniflorone L (X6, 9.7mg) (Boonnak *et al*., 2010).

Fraction B7 was chromatographed over a silica gel QCC eluted with EtOAc-*n*-hexane (30:70) to give 8 subfractions (B7A-B7H). Subfractions B7E and B7F were further purified on silica gel column eluting with EtOAc-*n*-hexane (30:70) to afford 20 sub fractions (B7E1-B7E20). Subfraction B7E8 was subjected to silica gel column eluted with a step gradient of acetone in *n*-hexane to give 1,7-dihydroxy-8-methoxyxanthone (X7, 17.5mg) (Kijjoa *et al*., 1998). Subfractions B7E10-B7E12 were fractioned on a silica gel QCC with a step gradient of $CH_2Cl_2$ in *n*-hexane to afford 12 subfractions (B7E10A-B7E10L). Sub fraction B7E10D was chromatographed over a silica gel QCC eluted with acetone-*n*-hexane (10:90) to afford 8 subfractions (B7E10D1-B7E10D8). Subfraction B7E10D5 was further subjected to silica gel column eluted with a step gradient of $CH_2Cl_2$ in *n*-hexane to give celebixanthone methyl ether (X8, 15.3mg) (Deachathai *et al*., 2006). Subfraction B7E10F was further purified over silica gel column eluted with a step gradient of acetone in *n*-hexane to give 8 subfractions (B7E10F1-B7E10F8). Subfraction B7E10F6 was subjected to silica gel column eluted with $CHCl_3$-*n*-hexane (60:40) to give 4 sub fractions (B7E10F6A-B7E10F6D) and a mixture of macluraxanthone (X11) and compound X12 (35.5mg) which was fractionated by reversed-phase silica gel chromatography eluting with 100% MeOH to obtain macluraxanthone (X11, 21.2 mg) and Garcinone B (X12, 12.0mg) (Sen *et al*., 1982). Fraction B8 was purified over silica gel column with a step gradient of acetone in *n*-hexane to give 4 fractions (B8A-B8D). Subfraction B8C was subjected to silica gel column eluted with a step gradient of acetone in *n*-hexane to give 3,4-dihydro-5,9-dihydroxy-7-(3-hydroxy-3-methylbutyl)-8-methoxy-2,2-dimethyl-2H,6H-pyrano[3,2-

b]xanthen-6-one (X17, 12.1mg) (Dutta *et al*., 1987) and pruniflorone A (X18, 32.2mg) (Boonnak *et al*., 2006).

***Acetylation of compound X15***

A mixture of compound X15 (15.0mg), $Ac_2O$ (0.25ml) and pyridine (1.0ml) was stirred at room temperature for 6h. After completion of the reaction, the resulting mixture was diluted with $H_2O$ and subsequence extracted with $CH_2Cl_2$ and the phases separated. The organic phase was acidified with 10% HCl and further dried over anhydrous $Na_2SO_4$. The resulting residue was purified over a silica gel column to furnish a mono-acetylated product (X16) (14.6mg). Compound X16: 6-acetoxy-*β*-mangostin (Jefferson *et al*., 1970) Pale yellow powder. For $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ (ppm): 13.16 (*s*, 1-OH), 7.02 (s, 1H), 6.26 (*s*, 1H), 5.15 (*m*, 2H), 4.07 (*d*, 2H, *J*=6.6 Hz), 3.83 (*s*,-$OCH_3$), 3.70 (*s*,-$OCH_3$), 3.28 (*d*, 2H, *J*=6.9 Hz), 2.32 (*s*, 6-OAc), 1.76 (*s*,-$CH_3$), 1.72 (*s*, -$CH_3$), 1.61 (*s*, 2 × -$CH_3$).

The chopped dried green fruits of *C. formosum* ssp. *pruniflorum* (5.5kg) were extracted for two times with $CH_2Cl_2$ (2 × 20L) by maceration at room temperature to give $CH_2Cl_2$ extract. The volume of combined $CH_2Cl_2$ extracts were reduced under vacuum to give a crude $CH_2Cl_2$ extract (31.42g), which was fractioned on a silica gel QCC eluting with a step gradient of acetone in *n*-hexane to obtain give 14 fractions (C1-C14). Fraction C4 was chromatographed over a silica gel column with a step gradient of acetone in *n*-hexane to afford 5 subfractions (C4A-C4E), a mixture of T1 (lupeol), T2 (*α*-amyrin) and T3 (*β*-amyrin) (>1.50g) and a mixture of *β*-sitosterol (T4) and stigma sterol (T5) (>45.6mg), respectively. A mixture of T1-T3 (60.2 mg) was purified successively by PLC eluting with $CH_2Cl_2$-*n*-hexane (10:90) to obtain Lupeol (T1, 13.1 mg) and a mixture of *α*-amyrin (T2) and *β*-amyrin (T3) (35.3mg) (Berrondo *et al*., 2003). Fraction C10 was chromatographed over a silica gel column a step gradient of acetone in *n*-hexane to yield 17 subfractions (C10A-C10Q). Subfractions C10N and C10O were further fractioned on silica gel column with a step gradient of EtOAc in *n*-hexane to obtain 8 subfractions (C10N1-C10N8). Compound X10, formoxanthone C (15.2mg) (Boonsri *et al*., 2006), was isolated from sub fraction C10N6 by CC eluted with $CH_3Cl$.

The chopped dried green fruits of *C. cochinchinense* (5.5 kg) were extracted for two times with $CH_2Cl_2$ (2 × 20L) by maceration at room temperature to give $CH_2Cl_2$ extract. The volume of combined $CH_2Cl_2$ extracts were reduced under vacuum to give a crude $CH_2Cl_2$ extract (40.04g), which was fractioned by QCC over a silica gel eluting with a step gradient of EtOAc in *n*-hexane to obtain 9 fractions (D1-D9). Fraction D6 was a mixture of X2 and X3 (3.01g), which was then chromatographed over a silica gel column with $CHCl_3$ to yield 6 sub fractions (D6A-D6F), cochinchinone E (X2, 490.2mg) (Mahabusarakam *et al*., 2008) and 7-geranyloxy-1,3-dihydroxyxanthone (X3, 2.10g) (Nguyen and Harrison, 1998). Compound X1, 1,3,7-trihydroxyxanthone (15.4 mg) (Laphookhieo *et al*., 2008), was isolated from fraction D7 by CC eluted with acetone-*n*-hexane (35:65).

***Bioassays***

*Antibacterial assay*

The isolated compounds from *Cratoxylum* plants were tested against the periodontopathic microorganism, *Porphyromonas gingivalis* (ATCC 33277). The antimicrobial assay employed was the agar diffusion technique. One hundred micro litre (µl) of BHI-suspended microorganism was distributed on the agar medium (25 ml/plate) using small-size glass beads. Once the agar surface was dried, paper discs 6 mm diameter (Whatman International, UK), soaked with 10µl of the isolated compound solution on each side of the disc, and placed on the agar surface. Chlorhexidine 2% and DMSO were used as positive and negative control respectively. Each plate was incubated in anaerobic chamber at 37°C in 5% $CO_2$ atmosphere for 7 days. All tests were performed in triplicate and the antibacterial activity was expressed as the mean diameters (mm) of inhibition zone produced by the isolated compound.

***DPPH assay***

The free radical scavenging activities of the test materials was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Standard solution was prepared (in the dark) by dilution of DPPH in absolute ethanol at 60, 50, 40, 30, 20 and 10µM prior to the test. The test samples were diluted to 1mg/ml. *L*-ascorbic acid (100 mg/ml) was used as positive control. 20µl of prepared test samples and *L*-ascorbic acid were put in clear polystyrene 96-well micro titer plate then 180µl of 60µM of DPPH were added to each well. The resulting solution was incubated in the dark for 30 minutes then the absorbance of the reaction mixture was determined using micro plate reader (Model series UV 900 Hdi, USA) at wavelengths of 515nm.

***Cytokine (IL-1β, TNF-α) production***

Each isolated compound (10 mg/ml) from different parts of *C. formosum* ssp. *pruniflorum* and *C. cochinchinense* was diluted to its non-cytotoxic concentration with RPMI1640 before treatment. The inhibitory effect of each compounds on the cytokine (IL-1β, TNF-α) production from the LPS-stimulated PMA-stimulated-monocytes was measured as described previously (Rodanant *et. al*., 2012). Supernatants were then collected and assayed for cytokines (IL-1β, TNF-α) by ELISA.

***Cytotoxicity assay***

The procedure for the cytotoxic assay was performed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method (Mosmann, 1983) with minor

**Table 1**: Antibacterial activity of isolated compounds in paper disc method against *Porphyromonas gingivalis*

| Isolated compounds[a] | Diameter of inhibition zone (mm) |
|---|---|
| X4+X5 | 14±0.87 |
| X5+X6 | 14±0.82 |
| X7 | 12±1.32 |
| A1 | 18±0.58 |
| A2 | 15±0.41 |
| A3 | 15±0.58 |
| T1+T2+T3 | 12±0.41 |
| Chlorhexidine 2% | 23±0.29 |
| Dimethylsulfoxide | 0 |

[a] 10 mg/ml

**Table 2**: Anti-oxidant activity of isolated compounds

| Compounds | Remaining of DPPH | Anti-oxidant activity (%) |
|---|---|---|
| X1 | 30.84 | 42.10 |
| X2 | 9.57 | 82.03 |
| X3 | 42.19 | 20.80 |
| X2+X3 | 38.64 | 27.45 |
| X4 | 32.97 | 38.10 |
| X5 | 35.63 | 33.11 |
| X4+X5 | 30.84 | 42.10 |
| X6 | 10.10 | 81.03 |
| X5+X6 | 50.34 | 5.49 |
| X7 | 45.02 | 15.47 |
| X8 | 51.58 | 3.16 |
| X9 | 10.64 | 80.03 |
| X10 | 27.47 | 48.42 |
| X11 | 32.44 | 39.10 |
| X12+X11 | 18.79 | 64.73 |
| X13 | 11.17 | 79.03 |
| X14 | 51.58 | 3.16 |
| X15 | 10.99 | 79.37 |
| X14+X15 | 43.25 | 18.80 |
| X16 | 18.97 | 64.39 |
| X17 | 49.99 | 6.16 |
| X18 | 40.24 | 24.46 |
| A1 | 53.00 | 0.50 |
| A2 | 55.83 | -4.83 |
| A3 | 56.54 | -6.16 |
| T1 | 45.55 | 14.48 |
| T2+T3 | 50.87 | 4.49 |
| T1+T2+T3 | 53.71 | -0.83 |
| T4+T5 | 53.53 | -0.50 |
| Ascorbic acid | 9.04 | 83.03 |

modification (Kuvatanasuchati *et al*., 2011). In this study, HGFs and monocytes (U937) were used. Each sample was measured in duplicate in two separate experiments.

## RESULTS

Since showing antimicrobial activity and has been used as traditional medicine for several purposes (Grosvenor *et al*., 1995a, Grosvenor *et al.*, 1995b, Kuvatanasuchati *et al*., 2011), crude extracts from *C. formosum* ssp. *pruniflorum* and *C. cochinchinense* were purified to yield secondary metabolites; anthraquinones, triterpenes and xanthones. These isolated compounds were subjected to test for their biological activities. Xanthone derivatives investigated in this study showed diverse activities. Different compounds exerted different activities. Two xanthone derivatives (X7 and a 1:1 mixture of X4 and X5) showed moderate antimicrobial activity against

**Table 3**: Effects of isolated compounds on cytokine release

| Compounds | Concentration of isolated compound (μg/ml) | IL-1β (pg/ml) | TNF-α (pg/ml) |
|---|---|---|---|
| X1 | 40 | 42.848 | >1000 |
| X3 | 20 | 98.065 | >1000 |
| X2+X3 | 20 | 87.196 | >1000 |
| X5 | 10 | Nil | Nil |
| X4+X5 | 20 | 16.326 | >1000 |
| X5+X6 | 10 | Nil | Nil |
| X7 | 20 | 81.978 | >1000 |
| X10 | 20 | 97.196 | >1000 |
| X14 | 10 | Nil | Nil |
| X14+X15 | 20 | 31.109 | >1000 |
| X16 | 20 | 34.152 | >1000 |
| X17 | 20 | 71.761 | >1000 |
| X18 | 50 | 9.587 | >1000 |
| A1 | 10 | 43.717 | >1000 |
| A2 | 20 | 40.022 | >1000 |
| A3 | 20 | 17.413 | >1000 |
| T1 | 30 | 159.587 | 48.773 |
| T2+T3 | 20 | 99.587 | >1000 |
| T1+T2+T3 | 50 | 65.457 | >1000 |
| T4+T5 | 20 | 63.283 | >1000 |
| Dexa | 0.5 | Nil | 7.864 |
| LPS | 1 | 58.283 | >1000 |
| RPMI | - | Nil | Nil |

studied gram-negative bacteria (table 1). Some compounds exhibited antioxidant effect comparable to ascorbic acid (X2, X6, X9, X13 and X15) (table 2). Only two compounds (X5 and X14) demonstrated a significant anti-inflammatory activity greater than dexamethasone (table 3) while possessing weak anti-oxidant activity and no antibacterial activity. Among all test materials, xanthones at 1:1 mixture of compounds X5 and X6 exhibited potent antibacterial and anti-inflammatory activities but, unfortunately, they were toxic to the U937 cells (table 4). Although anthraquinone fractions (A1-A3) did not exhibit anti-oxidant activity, they exerted the strongest antibacterial potential among all compounds (table 1). In term of anti-inflammatory activity, interesting inhibitory effects were observed on the expression of IL-1β, where compound A3 demonstrated the best result (table 3). No significant anti-inflammatory effect was shown by the triterpene compounds. Compound T1 seemed to be the most effective compound in the group. Though it demonstrated weak anti-oxidant effect, it was one among a few isolated compounds, which inhibited the release of TNF-α from the stimulated U937 (table 3). A 1:1:1 mixture of compounds T1, T2, T3 was the only triterpene fraction which showed weak-to-moderate inhibitory effect against *P. gingivalis* (table 1).

## DISCUSSION

The results from this study showed that isolated compounds from plant in *Clusiaceae* species and *Guttiferae* family demonstrated bactericidal effect on gram-negative microorganisms and DPPH scavenging activity which were consistent with previous studies (Sobral *et al*., 2009, Pretto *et al*., 2004; Tsaffack *et al*., 2009; Yahayu, 2013; Wansi *et al*., 2010, Zheng *et al*., 2011) but there were difference in potential efficacy.

Chemical structure of pure compounds seems to be an important factor determining their potential efficacy. From this study, core structure suggested its responsibility on the antibacterial activity; trioxygenated xanthone, dihydroxy anthraquinone and pentacyclic triterpene are shown to support this observation (table 1). Chemical compounds containing isoprene on position 2 and -OH group or $-OCH_3$ on position 3 were shown to exert the anti-oxidant and anti-inflammatory activity (table 2, 3 and fig. 1). This study showed that the difference of side chain on position 3 might have an effect on the potency of anti-inflammatory activity since compound X14 (contain -OH group on position 3) gave better inhibitory effect on the release of IL-1β than compound X16 (contain $-OCH_3$ group on position 3) (table 3 and fig. 1). Concerning the chemical structure, it seemed that compounds containing isoprenyl or geranyl or 1,1-dimethyl-2-propenyl on their side chain at position 4 and/or position 8 demonstrated cytotoxicity to U937 cells.

## CONCLUSION

All three chemical constituents (Anthraquinone, Xanthone, Triterpenes) showed diverse bioactivities.

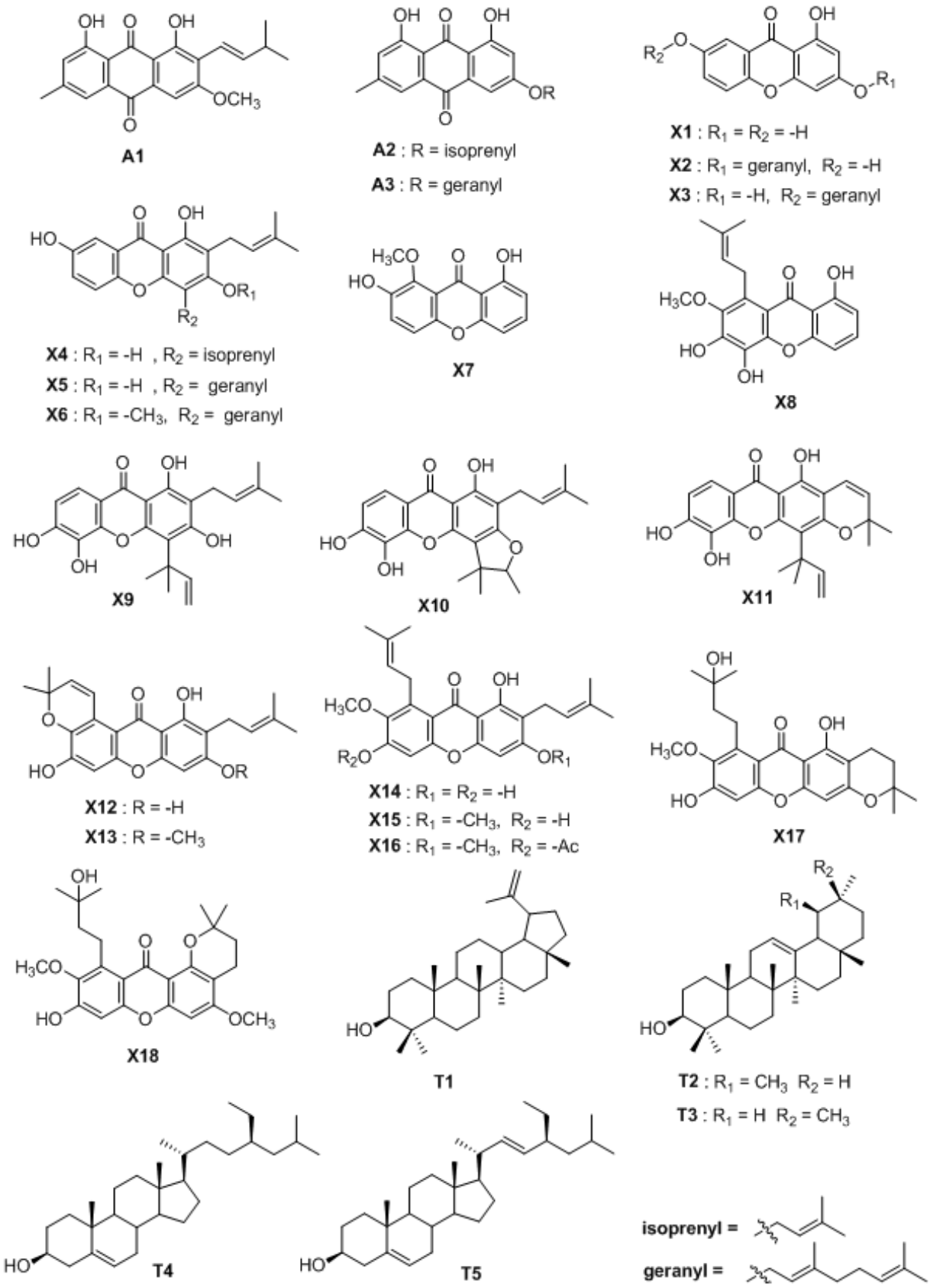


**Fig. 1**: Chemical structures of pure compounds

**Table 4**: *In vitro* cytotoxicity of isolated compounds from *C. formosum* ssp. *pruniflorum* and *C. cochinchinense* against U937 and human gingival fibroblasts (HGF)

| Compounds | $IC_{50}$ (µg/ml) | |
|---|---|---|
| | U937 | HGF |
| X1 | 58.56 | 270.97 |
| X2 | nil | 41.49 |
| X3 | 24.91 | 50.83 |
| X2+X3 | 33.01 | 43.99 |
| X4 | 10.79 | 41.19 |
| X5 | 21.49 | 77.32 |
| X4+X5 | 28.24 | 48.15 |
| X6 | nil | 45.38 |
| X5+X6 | nil | 36.39 |
| X7 | 28.03 | 50.92 |
| X8 | nil | 27.50 |
| X9 | nil | 30.59 |
| X10 | 35.09 | 68.61 |
| X11 | nil | nil |
| X12+X11 | nil | 21.16 |
| X13 | 1.22 | 32.38 |
| X14 | 28.04 | 41.72 |
| X15 | 5.57 | 55.89 |
| X14+X15 | 33.96 | 139.97 |
| X16 | 31.44 | 52.95 |
| X17 | 37.61 | 40.42 |
| X18 | 120.95 | $3.20 \times 10^6$ |
| A1 | 46.91 | 490.99 |
| A2 | 40.50 | 446.98 |
| A3 | 64.40 | $1.96 \times 10^5$ |
| T1 | $3.57 \times 10^3$ | 409.92 |
| T2+T3 | 120.04 | 685.26 |
| T1+T2+T3 | $8.06 \times 10^3$ | $4.22 \times 10^4$ |
| T4+T5 | 86.93 | 201.32 |

Since demonstration of potential result in antibacterial, anti-inflammatory and anti-oxidant activities, Xanthone (1:1 mixture of X4 (1,3,7-trihydroxy-2,4-diisoprenyl xanthone and X5 (cochinchinone A)) and Anthraquinone (A3 (3-geranyloxy-6-methyl-1,8dihydroxyanthraquinone) seem to represent promising primary source for developing effective medication in treating periodontal disease, an inflammatory disease provoked by periodontopathic bacterial accumulation.

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