INT. J. BIOL. BIOTECH., 20 (3): 417-421, 2023.

PHARMACOCHEMICAL STUDIES ON THE LEAVES OF *ENTANDROPHARGMA CANDOLLEI* HARMS

Aijaz Ali[1], [*]Muhammad Shaiq Ali[1], Mehreen Lateef[2] and Sidjui Sidjui Lazare[3]

[1]*H.E.J Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan*
[2]*Multi Disciplinary Research Lab, Bahria University Medical and Dental College, Karachi, Pakistan*
[3]*Institute of Medical Research and Medicinal Plant Studies, P.O Box 13033, Yaounde, Cameroon*
**Corresponding author's email: shaiq303@hotmail.com*

ABSTRACT

Pharmacochemical studies on the leaves of *Entandrophragma candollei* led to the isolation of seven secondary metabolites identified as gedunin (**1**), catechin (**2**), *β*-sitosterol (**3**), *β*-sitosterol glucoside (**4**), gallic acid (**5**), methyl gallate (**6**) and ethyl gallate (**7**), respectively. In addition, further 21 volatile constituents were identified *via* GCMS of the *n*-hexane fraction. The ethanolic extract, its subsequent sub-fractions and the pure isolates were subjected to biological screening to establish the scientific basis for the medicinal uses of the plant. Most of the tested samples were found to possess potent antioxidant and lipoxygenase inhibitory activities.

Key-words: *Entandrophragma candollei*, Meliaceae, phytochemistry, GC-MS, antioxidant activity, lipoxygenase inhibition

INTRODUCTION

The genus *Entandrophragma* (Meliaceae) is well known for its limoniods (Tchouankeu *et al*., 1989). The medicinal uses of the limoniods of various species of this genus include insecticidal, antibacterial, antifungal, antimalarial, and antiviral (Yenon *et al*., 2014; Tchouankeu *et al*., 1989). Apart from limonoids, other classes of secondary metabolites are also found in members of *Entandrophragma*. This includes sesquiterpenoids (Daniewski *et al*., 1996), flavonoids (Paula *et al*., 1997), acyclic terpenoids (Happi *et al*., 2015), phenols, steroids (Tchouankeu *et al*., 1996), alkaloids, tannins, quinones and cardiac-glycosides (Yenon *et al*., 2014). One of the species of this genus is *E. candollei* which is found in Cameroon and its neighbors, being used traditionally by indigenous people to treat a variety of diseases (Nnanga *et al*., 2016). Because of its strong antifeedant and antimalarial properties, the plant has also been widely used (Koul *et al*., 2003; Lusakibanza *et al*., 2010). Previously limonoids and tetranortriterpenoids have been reported from this species (Tchouankeu *et al*., 1989; Happi *et al*., 2021; Nnanga *et al*., 2016, Adesida *et al*., 1967 and Quasie *et al.*, 2016). We conducted pharmacochemical studies on *Entandrophargma candollei* due to its chemotaxonomic and ethnopharmacological significance.

MATERIALS AND METHODS

General experimental:

The chromatographic and spectroscopic protocols are similar to those recently reported (Maffo *et al*., 2023). GCMS was performed on a triple quad instrument (Agilent Technologies 7890A, linked with mass spectrometer, Jeol, JMS- 600H.

Collection of Plant Material:

Entandrophragma candollei Harms (Leaves) was collected in the month of October from Mont-Kala Yaoundé-Cameroon. It was authenticated by Mr. N.Victor, Cameroon National Herbarium (CNH). A voucher specimen of the collected plant material (No 1722/SRFK) has been deposited in the CNH.

Extraction and Isolation

Fresh leaves (20 kg) were dried in the shade, ground, and extracted three times with ethanol for a period of five days each. Vacuum Liquid Chromatography (VLC) was applied to separate the combined ethanolic extract (700 g) into various sub-fractions. The hexane soluble sub-fraction (80 g) was chromatographed over

silica gel, eluting with hexane-dichloromethane (DCM) mixtures in increasing order of polarity. The eluate from hexane-DCM (9:1) was an oily material which was subjected to GC-MS. *ß*-Sitosterol was obtained through elution with hexane-DCM (8:2) (Nnanga *et al*., 2016) (**3**, 6 mg). The ethyl acetate (EtOAc) sub-fraction from VLC (110 g) was chromatographed and eluted with mixtures of hexane-EtOAc in ascending order of polarity. This operation provided gedunin (Mitsui *et al*., 2006) [**1**, 7 mg; hexane-EtOAc (7.5:2.5)], methyl gallate (Ekaprasada *et al*., 2015) [**6**, 7 mg; hexane-EtOAc (6.3:3.7)], ethyl gallate (Sato *et al*., 1997) [**7**, 15 mg; hexane-EtOAc (6:4)], gallic acid (Sato *et al*., 1997) [**5**, 8 mg; hexane-EtOAc (5.5:4.5)], catechin (Jullian *et al*., 2007) [**2**, 10 mg; hexane-EtOAc (5:5)] and *ß*-sitosterol glucoside (Faizi *et al*., 2001) [**4**, 12 mg; hexane-EtOAc (4:6)]. The physical and spectral information for the aforementioned constituents demonstrated a strong agreement with those described in the literature, has been used to identify them.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

A GC capillary column [(HP-5 MS (5% phenylmethylpolysiloxane, 30 m, 250 m i.d., 0.25-m film thickness; Agilent, USA)] was used for the GCMS analysis. The oven temperature was set to 60°C for 5 min., then increased to 180°C at 3°C per min. for 10 min., and finally maintained at 300°C. The flow rate of the carrier gas helium was kept constant at 1 mL/min. The electron ionization source was used at 70 electron volts. The flow rate of the carrier gas helium was kept constant at 1 mL/min. The mass ranged from 50 to 700 amu. The identification of detected compounds was based on a comparison of individual compounds and their fragments with known compounds fed in the NIST Mass Spectrometry Data Centre (Mainlib.) NIST#: 352898 ID #: 113419 DB), USA.

Biological study

The crude ethanolic extract, its sub-fractions and pure isolates were subjected to extensive biological testing including inhibition studies against different enzymes. However, only notable results were observed in case of antioxidant activity and inhibitory potential against the enzyme lipoxygenase.

Determination of Antioxidant Activity

Oxidation is a process involving chemicals that result in the formation of free radicals which ultimately initiate chain reactions, leading to damage of the healthy cells. These undesirable reactions are regulated by the antioxidants. The antioxidative tendency of the tested samples was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Gülçin's method was applied to achieve DPPH radical-scavenging activity (Gülçin *et al.,* 2005).

Lipoxygenase (LOX) inhibition activity:

The activity of LOX is critical to the plant's defense against pathogens. This activity along with the resulting hydroperoxides of fatty acids, initiate free radical carbon chains, which induce protein modifications. The extract, sub-fractions and isolates have been tested for LOX using a modified spectrophotometric method developed by Tappel (1962).

RESULTS

The ethanolic extract of the leaves of *E. candollei* was subjected to VLC to obtain the sub-fractions soluble in hexane, dichloromethane, ethyl acetate and methanol, respectively. The chromatographic resolution of the hexane soluble sub-fraction resulted in the isolation and characterization of *β*-sitosterol (**3**). The ethyl acetate sub-fraction yielded six secondary metabolites namely gedunin (**1**), catechin (**2**), *β*-sitosterol glucoside (**4**), gallic acid (**5**), methyl gallate (**6**) and ethyl gallate (**7**) (Fig.1).

GCMS of the hexane sub-fraction resulted in the tentative identification of 21 further secondary metabolites which are described in Table 1.

Bio-screening of crude extract, sub-fractions and pure isolates

The DPPH free radical scavenging assay was used to assess the antioxidant activity of the crude ethanolic extract, its sub-fractions, and all pure isolates (Gülçin *et al.* 2005). The crude ethanolic extract and its hexane, dichloromethane, ethyl acetate and methanolic sub-fractions showed significant antioxidant activity (Table 2). On the other hand, crude ethanolic extract and its hexane and DCM sub-fractions were found to be moderately active against the enzyme lipoxygenase. On the other hand, ethyl acetate and methanolic sub-fractions showed potent activity (Table 2).

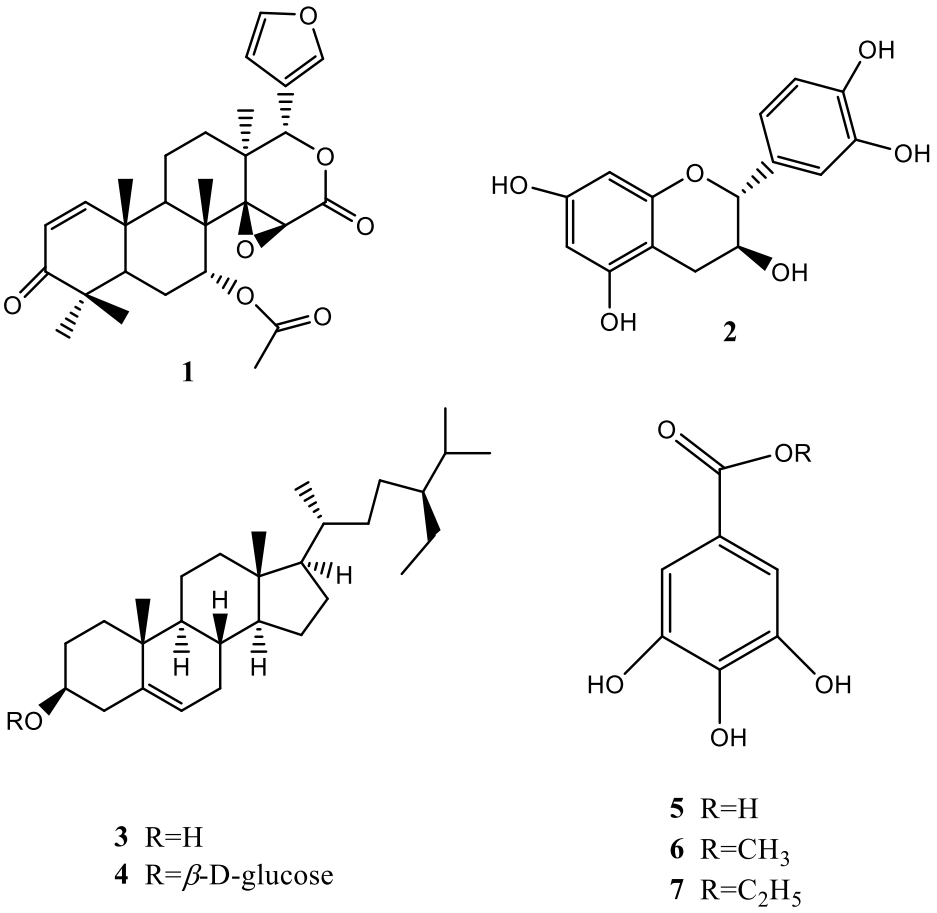

Fig.1. Structures of Isolates 1-7.

Table 1. Compounds Tentatively Identified from Hexane Fraction of *Entandrophragma. candollei.*

Peak #	**Ret.Time (Min.)**	**Name of Compound**	**%**
1	19.16	Octanoic acid	0.4
2	24.52	2,4-(*E*,*E*)-Decadienal	0.3
3	32.65	Ethyl 9-oxononanoate	1.4
4	35.98	Ethyl Dodecanoate	0.2
5	43.24	Myristic acid	0.8
6	48.05	Methyl hexadecanoate	1.3
7	52.43	Ethly hexadecanoate	34.7
8	60.88	Ethyl oleate	21.4
9	61.49	Ethyl stearate	4.1
10	63.80	Methyl-*cis*-10-nonadecenoate	4.3
11	70.10	Ethyl docosanoate	1.1
12	71.75	Ethyl eicosanoate	1.2
13	73.37	Ethyl tetracosanoate	2.2
14	73.88	Squalene	6.5

15	74.79	Tetratriacontane	2.1
16	75.44	Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15, 19-heneicosapentaenyl) (all-*E*)	0.6
17	75.62	Geranyl-geraniol	0.5
18	77.19	Cholesta-4,6-dien-3 *β*-ol	0.7
19	79.37	9,19-Cyclolanost-24-en-3β–*O*-acetate	0.4
20	79.82	1-Heptatriacotanol	0.9
21	80.89	13,27-Cyclours-3*β* –*O*-acetate	12.9
Total			**98.0**

Table 2. Biological Analysis of Extract and Fractions.

Extract, Fractions	**Antioxidant Activity %**	**Lipoxygenase Inhibition %**
Ethanol extract	**94.2 ± 0.14**	65.5 ± 0.33
Hexane fraction	**92.5 ± 0.63**	72.1 ± 0.12
Dichloromethane fraction	**96.3 ± 0.25**	69.8 ± 0.11
Ethyl acetate fraction	**92.4 ± 0.22**	**87.2 ± 0.77**
Methanol fraction	**93.2 ± 0.11**	**82.5 ± 0.14**
Std. BHA	**98.2 ± 0.06**	-
Baicalein	-	89.8± 0.08

Table 3. Biological Screening of Pure Isolates 1-7.

Isolate	**Antioxidant Activity IC_{50} (µM)**	**Lipoxygenase Inhibition IC_{50} (µM)**
Gedunin (**1**)	65.2 ± 0.49	85.9 ± 0.12
Catechin(**2**)	**21.0+0.11**	**18.9 ± 0.17**
β-Sitosterol(**3**)	N.A.	N.A.
β-Sitosterol glucoside(**4**)	N.A.	N.A.
Gallic acid(**5**)	**24.7+. 0.07**	**15.7 ±0.21**
Methyl gallate(**6**)	**25.8 ± 0.44**	**18.8 ± 0.36**
Ethyl gallate(**7**)	**26.2 ± 0.33**	**13.6 ± 0.21**
Std. BHA	44.2 ± 0.07	-
Baicalein	-	22.6 ± 0.08

N.A.: Not Active

Among the tested compounds, catechin, gallic acid, methyl gallate and ethyl gallate showed potent antioxidant activity (Table 3). The lipoxygenase inhibitory activity was determined using the method described by Tappel (Tappel, 1962). The same compounds also showed significant inhibitory potential against the enzyme lipoxygenase (Table 3), and these may serve as lead compounds towards the development of anti-inflammatory drugs.

DISCUSSION

A number of secondary metabolites have been isolated and characterized, followed by their detailed bio-screening to establish scientific basis of the medicinal uses of *E. candollei* (Koul *et al*., 2003 and Nnanga *et al*., 2016). Nevertheless, the isolates only displayed notable antioxidant activity and inhibitory potential against the enzyme lipoxygenase. It therefore appears that the medicinal uses of the plant may be due to the combined effects of these isolates.

ACKNOWLEDGEMENT

This research was undertaken at H.E.J. Research Institute of Chemistry, University of Karachi under the TWAS-ICCBS research exchange program for which S.S.L. is thankful to TWAS and ICCBS.

REFERENCES

Adesida, G.A. and D.A.H. Taylor (1967). The chemistry of the genus *Entandrophragma. Phytochemistry*, 6: 1429-1433.

Daniewski, W. M., W. Anczewski, M. Gumułka, W. Danikiewicz, U. Jacobsson and T.Norin (1996). Sesquiterpenoid constituents of *Entandrophragma cylindricum*. *Phytochemistry*, 43: 811-814.

Ekaprasada, M.T., H. Nurdin, S. Ibrahim and D. Dachriyanus (2015). Antibacterial activity of methyl gallate isolated from the leaves of *Toona sureni. Int. J. Adv. Sci., Engg. Inform. Tech*., 5: 280-282.

Faizi,S., M. Ali, R. Saleem and S. Bibi (2001). Complete ^{1}H and ^{13}C NMR assignments of stigma-5-en-3-*O*-*ß*-glucoside and its acetyl derivative**.** *Mag. Res. Chem*., 39: 399-405.

Gülçin,I., H.A. Alici and M. Cesur (2005). Determination of *in vitro* antioxidant and radical scavenging activities of propofol. *Chem. Pharm. Bull*., 53: 281-285.

Happi, G.M., S.F.Kouam, F.M.Talontsi, S. Zühlke, M. Lamshöft, and M. Spiteller (2015). Minor secondary metabolites from the bark of *Entandrophragma congoënse* (Meliaceae)**.** *Fitoterapia*, 102: 35-40.

Happi, G.M., G. P. M. Kemayou, H. Stammler, B. Neumann, M. Ismail, S.F. Kouam, J. D.Wansi, J.C Tchouankeu, M. Frese, B. N. Lenta and N. Sewald (2021). Three phragmalin-type limonoids orthoesters and the structure of odoratone isolated from the bark of *Entandrophragma candollei* (Meliaceae), *Phytochemistry*, (https://doi.org/10.1016/j.phytochem.2020.112537)

Jullian, C., S. Miranda, G. Zapata-Torres, F. Mendizábal and C. Olea-Azar (2007). Studies of inclusion complexes of natural and modified cyclodextrin with (+) catechin by NMR and molecular modeling**.** *Bioorg. Med. Chem*., 15: 3217-3224.

Koul, O., W.M. Daniewski, J.S. Multani, M. Gumulka and G.J. Singh (2003). Antifeedant effects of the limonoidis from *Entandrophragma candollei* (Meliaceae) on the gram pod borer, *Helicoverpa armigera* (Lepidoptera: Noctuidae). *J. Agri. Food Chem*., 51: 7271-7275.

Lusakibanza, M., G. Mesia, G., Tona, S. Karemere, A. Lukuka, M. Tits and M. Frederich (2010). *In vitro* and *in vivo* antimalarial and cytotoxic activity of five plants used in Congolese traditional medicine. *J. Ethnopharmacol*., 129: 398-402.

Maffo, T, D. N.D. Nganteng, M. Latif, P. Wafo and M. S. Ali (2023). Enzyme inhibitor secondary metabolites from *Canarium schweinfurthii* Engl. *Int. J. Biol. Biotech*. 20: 359-363.

Mitsui, K., H. Saito, R. Yamamura, H. Fukaya, Y. Hitotsuyanagi and K.Takeya (2006). Hydroxylated gedunin derivatives from *Cedrela sinensis***.** *J. Nat. Prod*., 69: 1310-1314.

Nnanga, N.G.A., V. Deli, F.N.L. Claire, S.S. Lazare, S. Sandrine, N.S. Arthur, T.K.R.Marie, N.D.Y. Oscar and M.M.Emmanuel (2016). Phytochemistry and *in vitro* antimicrobial, antioxydant activities of *Entandrophragma candollei* H. *J. App. Pharm. Sci*., 6: 73-79.

Paula, J. D., I.J.Vieira, J.B. Fernandes, P.C. Vieira, and E.F. Vilela (1997). Sesquiterpenes, triterpenoids, limonoids and flavonoids of *Cedrela odorata* graft and speculations on the induced resistance against *Hypsipyla grandella*. *Phytochemistry*, 44: 1449-1454.

Quasie, O., H. Li, J. Luo and L.Y. Kong (2017). Two new phragmalin-type limonoids orthoesters from *Entandrophragma candollei***.** *Chin. J. Nat. Med*., 15: 680-683.

Sato, Y., H. Oketani, K. Singyouchi, T. Ohtsubo, M. Kihara, H. Shibata and T. Higuti (1997). Extraction and purification of effective antimicrobial constituents of *Terminalia chebula* Retz. against methicillin-resistant *Staphylococcus aureus. Biol. Pharm. Bull*., 20: 401-404.

Tappel, A.L. (1962). Lipoxidase. *Methods in Enzymology*, 5: 539-542.

Tchouankeu, J.C., E. Tsamo, B.L. Sondengam, and J.D. Connolly (1989). Atomasins A and B, tetranortriterpenoids from the bark of *Entandrophragma candollei*. *Phytochemistry*, 28: 2855-2857.

Tchouankeu, J. C., B. Nyasse, E. Tsamo and J. D. Connolly (1996. 7α, 20 (*S*)-Dihydroxy-4, 24 (28)-ergostadien-3-one from *Entandrophragma utile*. *J. Nat. Prod.*, 59: 958-959.

Yenon, A., Y.F. Houphouet, K. A.Edwige, F.Y.Adou, D.N. Jean and D.D. Allico (2014). Acute toxicity and phytochemical screening of stem bark extracts of *Entandrophragma angolense* (Welw.) C.DC. (Meliaceae). *Asian J. Biomed. Pharm. Sci*., 4: 50-56.

(*Accepted for publication June 2023*)