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RESEARCH ARTICLE

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SYNTHESIS AND ANTIOXIDANT ACTIVITY OF GALLOYLTYROSINE, DERIVATIVES FROM YOUNG LEAVES OF *INGA LAURINA*.

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ABSTRACT

Synthesis of Galloyltyrosine and its derivatives **9a-e** and **13a-c** was achieved from appropriately substituted benzoic acid and L-tyrosine. While compounds **9a-e** and **13a-c** was exhibited potent antioxidant activity in both the NBT and DPPH – radical scavenging models among the synthesized Galloyltyrosine derivatives.

KEY WORDS

Inga laurina, synthesis, Galloyltyrosine,Antioxidant properties, DPPH assay.

INTRODUCTION

Galloyl depsides of Tyrosine are widely distributed in the young leaves of *Inga laurina* and the other galloyl group derivatives presented in the leaves of *Koelreuteria Paniculata Laxm* are known to exhibit a wide range of pharmacological activities such as anti-fungal and anti-bacterial agents [1].Recently ,John Lokvan et al., isolated few Galloyl Depsides of Tyrosine from young leaves of *Inga laurina* [2]. Since we are interested on the antioxidant activity of natural phenolic secondary plant metabolites especially galloyl group derivatives[3] .Here we would like to report the synthesis and antioxidant activity studies on the synthesized galloyl tyrosine derivatives **9a-e** and **13 a-c** for the first time.

MATERIALS AND METHODS

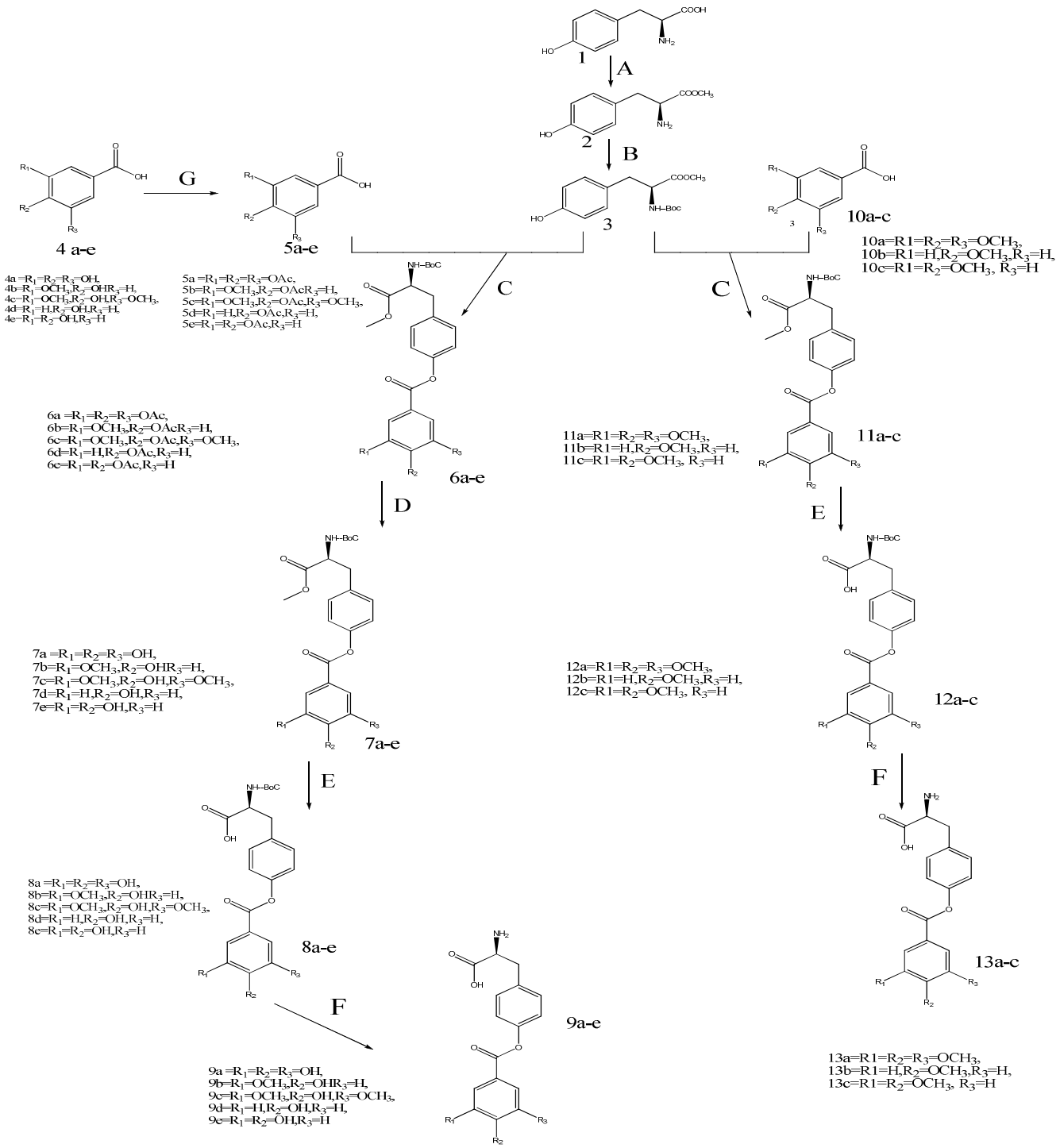
Experimental Section

Melting points were recorded on a V Scientific melting point apparatus, in open capillaries and are uncorrected. IR spectra were recorded on a Perkin-Elmer BX1 FTIR Spectrophotometer, ^{1}HNMR (400 MHz) & ^{13}C NMR (100 MHz) spectra on a Bruker 400 MHz NMR spectrometer and the values for chemical shifts (δ) being given in ppm and coupling constants (J) in Hertz (Hz). Mass spectra were recorded on Agilent 1100 Series LC/MSD .All the reactions were monitored by thin layer chromatography (TLC) using precoated silica 60 F254, 0.25 mm aluminum plates (Merck). Column chromatography was carried out using ACMEsilica gel (100-200 mesh/finer than 200 mesh). All the chemicals and solvents used were of synthetic grade and were purified prior to use.

General Procedure of the synthesis

The desired Galloyltyrosine and It's derivatives were synthesized as shown in **scheme – 1** starting from substituted benzoic acid **4a-e** and L-tyrosine. Compound **1**, which was esterification with $SOCl_2$ and MeOH to furnish compound **2** [4]. Selective Boc protection of compound **2** with $(Boc)_2O$ and $NaHCO_3$ in water, THF at rt for 1hr,gave compound **3**, O-Acetylation of compound **4a-e** using Acetic anhydride in Pyridine gave compound **5a-e**. Condensation of **5a-e** with compound **3** using DCC [5] as dehydrations agent in presence of DMAP (dimethyl amino pyridine) as catalyst in dichloro ethane (EDC) to give compound **6a-e**. Compound **6 a-e** was treated with $NaHCO_3$ and MeoH to obtain compound **7a-e**.Treatment of compound **7a-e** with LiOH and1,4-Dioxane Yielded compound **8a-e** [6] . Compound **8a-e** was treated with 1,4-Dioxane followed by de boc protection with TFA in MDC to afford **9a-e [7]** (**Scheme – I**). Condensation of **10a-c** with compound **3** using DCC as dehydrating agenet in presence of DMAP as catalyst in dichloro ethane (EDC) to give compound **11a-c**. Treatment of compound **11a-c** with LiOH and 1,4-Dioxane Yielded compound **12a-c**.Compound **12 a-c** was treated with 1,4-Dioxane followed by de Boc protection with TFA in MDC to afford **13 a-c** (**scheme – I**).

SCHEME - I


1
A
2
B
3
G
4 a-e
5a-e
10a-c
10a=R1=R2=R3=OCH3,
10b=R1=H,R2=OCH3,R3=H,
10c=R1=R2=OCH3, R3=H
4a=R1=R2=R3=OH,
4b=R1=OCH3,R2=OHR3=H,
4c=R1=OCH3,R2=OH,R3=OCH3,
4d=R1=H,R2=OH,R3=H,
4e=R1=R2=OH,R3=H
5a=R1=R2=R3=OAc,
5b=R1=OCH3,R2=OAcR3=H,
5c=R1=OCH3,R2=OAc,R3=OCH3,
5d=R1=H,R2=OAc,R3=H,
5e=R1=R2=OAc,R3=H
C
C
6a=R1=R2=R3=OAc,
6b=R1=OCH3,R2=OAcR3=H,
6c=R1=OCH3,R2=OAc,R3=OCH3,
6d=R1=H,R2=OAc,R3=H,
6e=R1=R2=OAc,R3=H
6a-e
11a=R1=R2=R3=OCH3,
11b=R1=H,R2=OCH3,R3=H,
11c=R1=R2=OCH3, R3=H
11a-c
D
E
7a=R1=R2=R3=OH,
7b=R1=OCH3,R2=OHR3=H,
7c=R1=OCH3,R2=OH,R3=OCH3,
7d=R1=H,R2=OH,R3=H,
7e=R1=R2=OH,R3=H
7a-e
12a=R1=R2=R3=OCH3,
12b=R1=H,R2=OCH3,R3=H,
12c=R1=R2=OCH3, R3=H
12a-c
E
F
8a=R1=R2=R3=OH,
8b=R1=OCH3,R2=OHR3=H,
8c=R1=OCH3,R2=OH,R3=OCH3,
8d=R1=H,R2=OH,R3=H,
8e=R1=R2=OH,R3=H
8a-e
F
9a-e
13a-c
9a=R1=R2=R3=OH,
9b=R1=OCH3,R2=OHR3=H,
9c=R1=OCH3,R2=OH,R3=OCH3,
9d=R1=H,R2=OH,R3=H,
9e=R1=R2=OH,R3=H
13a=R1=R2=R3=OCH3,
13b=R1=H,R2=OCH3,R3=H,
13c=R1=R2=OCH3, R3=H

Reagents and Conditions: (A) $SOCl_2$, MeOH, rt, 3.5hrs (B) $(Boc)_2O$,$NaHCO_3$ H_2O:THF(1:1) rt ,1h, (C) DCC ,DMAP,EDC ,rt,4hrs (D) $NaHCO_3$,MeOH, rt,1h,
(E) LiOH ,1,4- Dioxane ,rt,1h, (F) TFA ,MDC ,rt,2hrs, (G) AC_2O,Pyridine,rt,4hrs.

Preparation of (S)-methyl 2-amino-3-(4-hydroxyphenyl)propanoate (2):
L-Tyrosine was dissolved in MeOH and added $SOCl_2$ by drop wise at 0-5^0 C and the reaction mixture was stirred at room temperature for 3 to 4 hrs .After completion of starting material MeOH was concentrated under reduced pressure and washed with n-Hexane and dried under vacuum obtained as pale yellow colored solid.
m.p 134-136^0 C ; IR (KBr cm^{-1}): 3357,2813,1744,1597, ^{1}HNMR (400 MHz, DMSO): δ 9.29 (s, 1H, OH),6.95-6.93 (d, 2H-Ar-H), 6.66-6.64 (d, 2H, Ar-H), 3.56(s, 3H, CH_3), 3.46(t,1H,CH),2.76-2.63 (d, 2H, CH_2).,LCMS (Positive Mode): 196 $[M+H]^+$.

General procedure for the O-Acetylation (5a-e):
Hydroxy substutied benzoic acid(4a-e) was dissolved in Pyridine as a solvent to this 2.5 e.qt of AC_2O was added at room temperature and stirred at same temperature for over night.After completion of starting material the reaction mixture was poured into ice cold water ,acidify with dil.HCl and stirred for 15 mins,filtered under vacuum ,washed with cold water and dried .m.p 354-356^{o}C.

Condensation Procedure for (6a-e) and (11a-c) :
To a stirred solution of (5a-e) and (10a-c) in EDC was added DCC in EDC and stirred for about 15 mins. At r.t. To the above reaction mixture was added NH-Boc protected methyl 2-amino-3-(4-hydroxyphenyl)propanoate (3) fallowed the addition of catalytic amount DMAP and continued to stir at r.t for further 1h. DHU formed was filtered and filtrate was poured into water ,extracted with EtOAC . The combined organic layer was washed with water ,brine and dried over anhydrous Na_2SO_4 .The crude obtained by the evaporation of the solvent was chromatographed using chloroform and methanol as the eluent (90:10) to yield corresponding products **(6a-e) and (11a-c).**

DeProtection of acetyl groups (7a-e) :
To a stirred solution of (6a-e) in MeOH and added $NaHCO_3$(6.0 m.mol) at room temperature and stirred for further 1h.After completion of starting material the reaction mixture was filtered under vacuum to remove unreacted $NaHCO_3$ then the methanolic layer was poured into ice cold water and stirred for 15 mins filtered over vacuum and washed with ice cold water and dried under vacuum to get the compound**(7a-e)**.

Spectral data of the compounds (9a-e) and (13a-c):
3-(4-(3,4,5-trihydroxybenzoyloxy)phenyl)-2-aminopropanoic acid (9a):
Yield 62% ; White powder m.p 236-238^{0}C IR (KBr cm^{-1}): 3428,1708,1620,1504,1450,1396, 1344, 1310, 1216, 1203; ^{1}H NMR (DMSO, 400 MHz) δ 8.28(br,s,1H), 7.39 (d,2H),7.10 (d,2H),7.09 (s,2H) 3.67(dd,1H), 3.30 (dd,1H),3.01 (dd,1H), LCMS (Negative Mode): 332 $[M-H]^-$.

3-(4-(4-hydroxy,3-methoxybenzoyloxy)phenyl)-2-aminopropanoic acid (9b): Yield 68% ; Off white powder m.p 242-244^{0}C, IR (KBr cm^{-1}): 3421, 2830,1710,1650,1508,1446,1390, 1344, 1310, 1216, 1203; ^{1}H NMR (DMSO, 400 MHz) δ 9.08(br,s,1H),7.37(d,2H),7.30(s,1H),7.29(d,1H), 7.26(d,1H),7.15(d,2H) 3.79(dd,1H), 3.50(s,3H),3.35(dd,1H),3.23(dd,1H), LCMS (Positive Mode): 332 $[M+H]^+$.

3-(4-(4-hydroxy,3,5-dimethoxybenzoyloxy)phenyl)-2-aminopropanoic acid (9c): Yield 73% *;* Brown powder m.p 267-269^{0}C, IR (KBr cm^{-1}): 3418, 2832,1716,1653,1505,1440,1386, 1341, 1313, 1220, 1208; ^{1}H NMR (DMSO, 400 MHz) δ8.08(br,s,1H),7.35(d,2H),7.30(s,2H),7.15(d,2H) ,3.79(dd,1H), 3.48(s,6H),3.32(dd,1H),3.18(dd,1H), LCMS (Positive Mode): 362 $[M+H]^+$.

3-(4-(4-hydroxybenzoyloxy)phenyl)-2-aminopropanoic acid (9d): Yield 54% ; White powder m.p 226-228^{0}C, IR (KBr cm^{-1}): 3426, 1710,1651,1509,1443,1380,1341,1208; ^{1}H NMR (DMSO, 400 MHz) δ8.28(br,s,1H),7.40(d,2H),7.38(d,2H),7.37(d,2H),7.15(d,2H),3.75(dd,1H), 3.32(dd,1H),3.21(dd,1H), LCMS (Negative Mode): 300 $[M-H]^-$.

3-(4-(3,4-dihydroxybenzoyloxy)phenyl)-2-aminopropanoic acid (9e): Yield 48% ; White powder m.p 221-222^{0}C, IR (KBr cm^{-1}): 3424, 1712,1642,1509,1443,1389,1341,1310,1218; ^{1}H NMR (DMSO, 400 MHz) δ8.38(br,s,1H),7.38(d,2H),7.32(d,1H),7.28(s,1H) ,7.25(d,1H),7.15(d,2H),3.73(dd,1H), 3.34(dd,1H),3.25(dd,1H), LCMS (Negative Mode): 316 $[M-H]^-$.

3-(4-(3,4,5-trimethoxybenzoyloxy)phenyl)-2-aminopropanoic acid (13a): Yield 78% ; Pale yellow powder m.p 302-304^{0}C, IR (KBr cm^{-1}): 3418, 2840,1730,1646,1513,1446,1342,1216,1202; ^{1}H NMR (DMSO, 400 MHz) δ 7.40(d,2H),7.29(s,2H),7.18(d,2H),3.82(s,6H),3.7 7(dd,1H),3.51(dd,1H)3.45(dd,1H),3.32(s,3H), LCMS (Positive Mode): 376 $[M+H]^+$.

3-(4-(4-methoxybenzoyloxy)phenyl)-2-aminopropanoic acid (13b): Yield 86% ; Yellow powder m.p 294-296^{0}C, IR (KBr cm^{-1}): 3420, 2847,1724,1626,1503,1440,1340,1210,1200; ^{1}H NMR (DMSO, 400 MHz) δ 7.39(d,2H),7.29(d,2H),7.25(d,2H),7.20(d,2H),3. 73(dd,1H),3.67(dd,1H),3.51(s,3H),3.35(dd,1H), LCMS (Positive Mode): 316 $[M+H]^+$.

3-(4-(3,4-dimethoxybenzoyloxy)phenyl)-2-aminopropanoic acid (13c): Yield 88% ; Pale yellow powder m.p 346-348^{0}C, IR (KBr cm^{-1}): 3410, 2836,1718,1632,1508,1442,1337,1210,1204; ^{1}H NMR (DMSO, 400 MHz) δ 7.41(d,2H),7.32(d,1H),7.27(s,2H),7.24(d,1H),7.2 0(d,2H),3.91(dd,1H),3.76(s,3H),3.65(dd,1H),3.5 5(s,3H)3.44(dd,1H), LCMS (Positive Mode): 346 $[M+H]^+$.

RESULTS AND DISCUSSION

The above synthesized Galloyl tyrosine derivatives were evaluated for their antioxidant activities. Antioxidant potency was carried out in both the DPPH [8]free radical inhibition and super oxide radical inhibition (NBT) methods [9]. Galloyl tyrosine and it's derivatives exhibited significant antioxidant activity both in DPPH and NBT free radical inhibition models with IC_{50} values.The anti oxidant activities of both the series 9(a-e) and 13(a-c) respectively, have been carried out . The results show that the some of the prepared compounds exhibited excellent activity when compared to standards. Since the anti oxidant activity effect has been attributed to the presence of a hydroxyl groups, it is of interest to study effect of fixation of these groups to the Galloyltyrosine moiety. The compounds 9a, 9b, 9c, 9d, 9e and 13b exhibited excellent activity when compared to standard vitamin-c in DPPH method and The compounds 9a, 9b, 9e and 13b exhibited excellent activity when compared to standard gallic acid in NBT method.

Table I
Anti oxidant activity of compounds (9a-e) and (13a-c)

Compound	IC_{50} µg/ml	
	NBT method	DPPH method
9a	0.55	1.55
9b	0.76	2.64
9c	1.56	3.48
9d	1.78	2.31
9e	0.63	1.89
13a	5.64	8.63
13b	0.69	4.29
13c	4.32	6.83
Vitamin-C	-	4.29
Gallic acid	0.65	-

CONCLUSION

This clearly indicates the importance of hydroxyl group (s) on both DPPH and NBT to exhibited better antioxidant activity. The present study highlights the importance of hydroxyl substituent's responsible for anti oxidant activities and therefore may serve for further modification to obtain clinically useful novel entities.

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