s, OMe-3'), 3.80 (3H, s, OMe-4'), 3.88 (3H, s, OMe-3), 5.06–5.15 (2H, m, H-9'), 5.61 (1H, br s, OH-4), 5.82 (1H, s, H-5'), 5.85–6.00 (1H, m, H-8'), 6.15 (1H, s, H-2'), 6.77–6.91 (3H, m, H-2, H-5 and H-6) and 6.94 (1H, s, H-7). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.0 (C-9), 32.7 (C-7'), 52.5 (OMe-3'), 55.8 (OMe-3), 56.1 (OMe-4'), 79.9 (C-3'), 105.7 (C-5'), 111.8 (C-2), 114.0 (C-5), 117.0 (C-9'), 122.2 (C-6), 127.1 (C-7), 129.6 (C-1), 132.2 (C-8), 134.9 (C-8'), 139.5 (C-1'), 141.2 (C-2'), 144.4 (C-4), 146.0 (C-3), 172.3 (C-4') and 187.0 (C-6').

Crotepoxide (10)

White crystal (4.2 g), mp 146°C-148°C, $[\alpha]_D^{26}$ + 190.4 (c 1.00, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 1754, 1727, 1451, 1373, 1275, 1236, 1120, 1068, 1045, 903, 719; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.99 (3H, s, OAc-3), 2.12 (3H, s, OAc-4), 3.05 (1H, dd, J 1.5, 2.2 Hz, H-2), 3.38 (1H, dd, J 2.2, 3.7 Hz, H-1), 3.60 (1H, d, J 2.2 Hz, H-7), 4.16 (1H, d, J 12.1 Hz, H-6a), 4.54 (1H, d, J 12.1 Hz, H-6b), 4.94 (1H, dd, J 1.1, 7.5 Hz, H-3), 5.64 (1H, d, J 9.0 Hz, H-4), 7.47 (2H, t, J 7.5 Hz), 7.62 (1H, t, J 7.5 Hz) and 8.02 (2H, d, J 7.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.6 (C-Ac-CH3), 48.0 (C-1), 52.5 (C-2), 53.8 (C-7), 59.4 (C-5), 62.4 (C-6), 69.5 (C-4), 70.4 (C-3), 128.5, 129.2, 129.8, 133.4, 165.6, 169.5 and 169.9 (CO-Ac).

Free radicals scavenging and antioxidant activities

2, 2'-Azinobis (3-ethyl benzthiazoline-6-sulphonic acid) is oxidized to its radical cation $ABTS^+$ by reaction with potassium persulphate[17] and represent peroxyl radicals. The $ABTS^+$ cation is intensely colored, and the ability of test material to reduce its color represents radical scavenging capacity. $ABTS^+$ cation is soluble in both aqueous and organic solvents and is not affected by ionic strength, so can be used in multiple media to determine both hydrophilic and lipophilic antioxidant capacities of extracts or body fluids.[18] The $DPPH^·$ radical is one of the few stable organic nitrogen radicals, which bears a deep purple color. It is commercially available and does not have to be generated before assay like $ABTS^+$. This assay is also based on measurement of reducing ability of antioxidants toward $DPPH^·$ radical. Both the $ABTS^+$ and $DPPH^·$ tests are simple to adapt and rapid in performing analyses. Hence, they are widely used in antioxidant screening. Ohlyan *et al.*[19] have identified the presence of $ABTS^+$ scavenging activity and anticancer properties in various extracts of fruits of *P. attenuatum*. Our study finds that chloroform extract of its fruit bears potent antioxidant activity and identifies that Neolignans present in this extract are potent molecules for $ABTS^+$ cation scavenging. However, only Piperkadsin A (9) could scaveng $DPPH^·$ radical. Figure 2a and b represents concentration dependent ABTS and DPPH radical scavenging

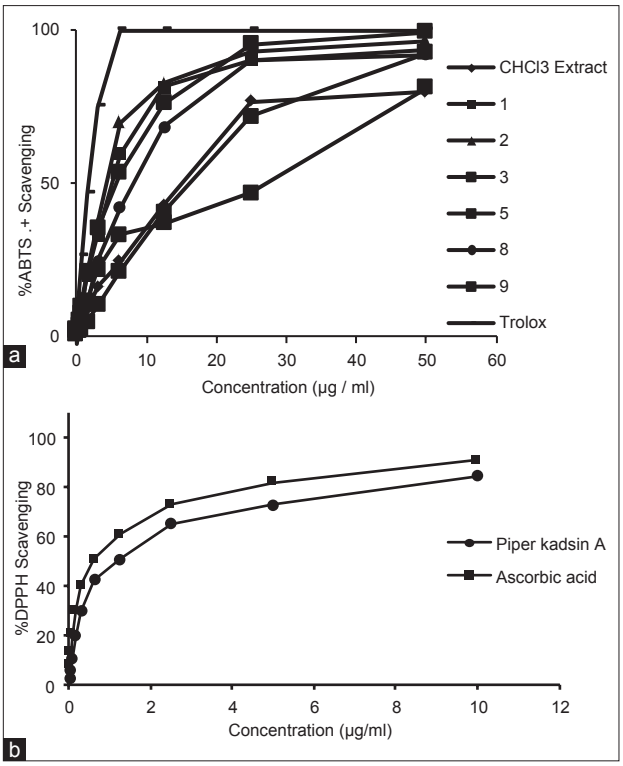

Figure 2: (a) Concentration dependent 2, 2'-Azinobis (3-ethyl benzthiazoline-6-sulphonic acid)+ scavenging pattern of chloroform extract of *Piper attenuatum* fruits and compounds isolated from the extract (b) Concentration dependent 2, 2-Diphenyl-1-picrylhydrazyl free radical scavenging pattern of piper kadsin A (9)

potential of the compounds. Evaluation of free radical scavenging concentration SC_{50} [Table 1] revealed that all the neo-lignans displayed the $ABTS^+$ radical scavenging activity, but only Piperkadsin A (9) could display $DPPH^·$ scavenging activity [Table 1]. The results indicated that the presence of methoxyl groups in either of the rings significantly affected compounds $ABTS^+$ radical scavenging potential as absence of methoxyl groups in crotepoxide (10) drastically reduced its $DPPH^·$ and $ABTS^+$ radical scavenging capacity.

Hyperglycemia is known to induce oxidative stress and increased generation of free radicals in the body.[20] For compound present in chloroform extract displayed potent free radical scavenging activity, we also studied its effect on glycemic activity by oral administration following oral glucose tolerance test in rats.[21] The results indicated that before glucose feeding could not influence glycemic values over time [Figure 3a] and overall 2 h glycemic load [Figure 3b] in rats.

In summary, the nine neolignans (1-9) were isolated and identified for the first time from *P. attenuatum* fruits and displayed potent $ABTS^+$ scavenging activity. Involvement of furan ring and R-configuration of methoxy groups present

Table 1: Concentrations required for scavenging ABTS$^+$ and DPPH radicals by 50% (SC_{50}) by the extract and compounds isolated from *Piper attenuatum*

Compound	ABTS$^+$ (SC_{50}, μM)	DPPH (SC_{50}, μM)
$CHCl_3$ extract	13.35 (μg/mL)	NA
Denudatin B (1)	13.10	NA
iso-4´, 5´-dimethoxy-3, 4-methylen edioxy-2´-oxo-$\Delta^{3',5',8'}$-8.1´-lignan (2)	11.22	NA
Lancifolin D (3)	56.64	NA
Wallichinine (5)	40.99	NA
2-oxo-piperol B (8)	20.14	NA
Piperkadsin A (9)	14.73	2.99
Trolox	6.47	ND
Ascorbic acid	ND	3.34

DPPH: 2, 2-Diphenyl-1-picrylhydrazyl; ABST: 2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; NA: Not active; ND: Not determined

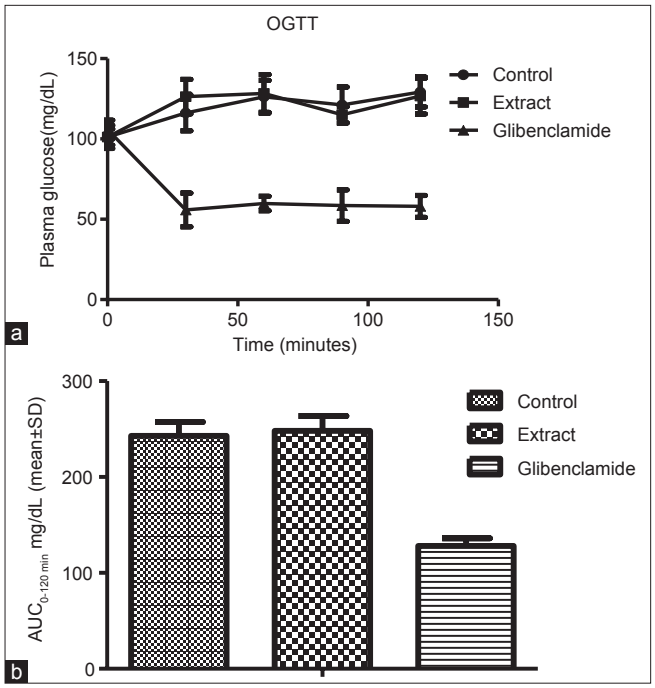

Figure 3: (a) Influence of chloroform extract of *Piper attenuatum* on postprandial plasma glucose level of rats following oral glucose tolerance test. 250 mg/kg body weight dose of extract and 5 mg/kg-body weight dose of standard antihyperglycemic drug glibenclamide was used in the study (b) Two hours glycemic loads under Influence of chloroform extract of *Piper attenuatum* following oral glucose tolerance test in normal rats. Area under the curve (mg/dL/hr) represent per hour postprandial glycemic load. ANOVA followed by Dunnett's multiple comparison tests was applied to compare difference between the groups

in neolignans were found major players in influencing ABTS$^+$ scavenging potentials. Piperkadsin A (9) displayed potent DPPH$^\cdot$ scavenging activity due to the presence of OH-group on benzene ring. The antioxidant rich chloroform extract of fruits of *P. attenuatum* did not display antihyperglycemic activity in rats after glucose feeding.

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