Research Journal of
Phytochemistry

ISSN 1819-3471

Academic
Journals Inc.

www.academicjournals.com

Research Journal of Phytochemistry 1 (1): 1-11, 2007
ISSN 1819-3471

Phytochemical Investigation and Biological Evaluation of *Schinus terebinthifolius*

[1]Amal M. Youssef Moustafa, [2]Simeon F. Kouam, [3]Aisha Kulsoom, [3]Asma Ejaz, [3]Shamsher Ali, [3]Shazia Anjum and [3]M. Iqbal Choudhary
[1]Department of Chemistry, Faculty of Science, Suez Canal University, BP 41522, Ismailia, Egypt
[2]Department of Chemistry, Higher Teachers' Training College, University of Yaounde 1, BP 47, Yaounde, Cameroon
[3]H.E.J. Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi, Karachi 75270, Pakistan

Abstract: Alcoholic extract of the aerial parts of *Schinus terebinthifolius* exhibit significant antioxidant, antifungal, antialzheimer`s and antileishmanicidal activities. Investigation of the chemical constituents of this plant let to isolate one new naturally occurring compound, synthetically known named (4-aminophenyl) acetic acid, along with the known 2-phenylacetamide, 1-pentadecanol, 3-(4-aminophenyl) prop-2-enoic acid, (*E*), ethyl 3, 4, 5-trihydroxybenzoate, cinnamic acid and benzamide. The structures of these compounds were established by spectroscopy techniques, including 1D and 2D NMR spectroscopy and comparison with the published data. The structure of (4-aminophenyl) acetic acid has also been confirmed by X-ray diffraction studies. The total alcoholic extract of *Schinus terebinthifolius* was evaluated for several bioassay activities and the isolated compounds were evaluated for their antifungal and antioxidant activities.

Key words: *Schinus terebinthifolius*, Anacardiaceae, antifungal activity, antioxidant activity, p-aminobenzyl acetic acid

INTRODUCTION

The Anacardiaceae includes 76 genera with over 600 species. A survey of the literature reveals that 25 of those genera contain poisonous species (Mitchell, 1990).

Schinus terebinthifolius RADDI (COPAL) is an ornamental plant, which belongs to family Anacardiaceae, genus *Schinus*. It is known as pink peppercorn (Jain *et al*., 1995), Brazilian pepper tree (Ronald, 1999), Pepper tree, Christmas berry, Faux Poirier, Florida Holly and Warui (Morton, 1978; Williams *et al*., 2002).

Uses in Traditional Medicine

Antihemorrhagic (reduces bleeding), analgesic (pain-reliever), antiinflammatory, antibacterial, anticancerous, anticandidal, antifungal, antispasmodic, antitumorous, antiviral, laxative, astringent, digestive stimulant, tonic, cardiotonic, hypotensive, wound healer, to stop bleeding and for toothaches. It is taken internally for rheumatism and as a purgative (Sarita Varma, 2002). It is used for many conditions in the tropics, including menstrnal disorders, bronchitis, gingivitis, gonorrhea, gout, eye infections, sores, swellings, tuberculosis, ulcers, urethritis, urogenital disorders, venereal diseases and warts. It is also used for colds, flu and other upper respiratory infections (Lloyd *et al*., 1977).

Corresponding Author: Amal M. Youssef Moustafa, Department of Chemistry, Faculty of Science, Suez Canal University, BP 41522, Ismailia, Egypt Tel: 002066-3727487

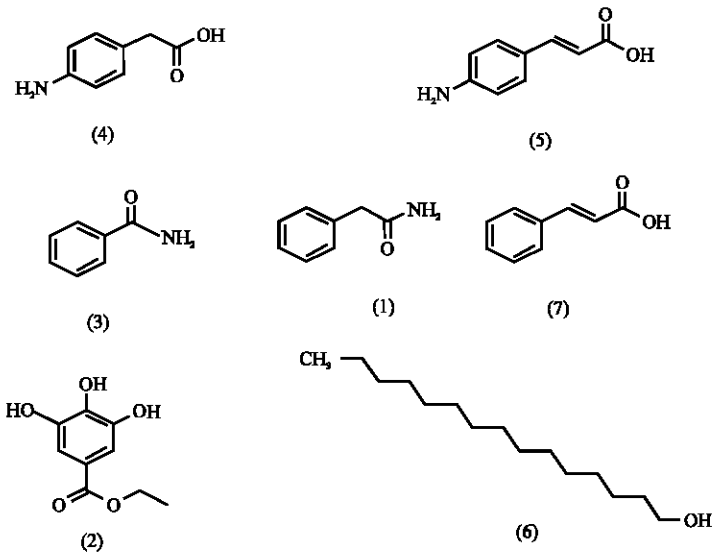

Fig. 1: Structures of isolated compounds

Previously Isolated Constituents

Phytochemical analysis of Brazilian pepper tree reveals that the plant contains tannins, alkaloids, flavonoids, steroidal saponins, sterols, terpenes and a large amount of essential oil (Lloyd *et al*., 1977; Stahl *et al*., 1983; Skopp and Schwenker, 1984; Campello and Marsaioli, 1974; 1975; Kaistha and Kier, 1962b; Hayashi *et al*., 1990).

New Isolated Constituents

New naturally occurring and synthetically known (4-aminophenyl) acetic acid (Schwartz *et al*., 1987), 2-phenylacetamide (Giridhar *et al*., 2003, Manley and Bilodeau, 2004, Firouzabadi *et al*., 1998, Guranda *et al*., 2001; Peng *et al*., 2003), 3-(4-aminophenyl) prop-2-enoic acid, (*E*) (Aleksi *et al*., 2001; Ono *et al*., 1999; Shingo *et al*., 2003), cinnamic acid (Marco *et al*., 1978; Ripperger *et al*., 1981), benzamide (Persinos *et al*., 1967; Douglas *et al*.,1997; Buller *et al*., 1992; Lord *et al*., 1973; Cook, 1989), Ethyl gallate (Mehta *et al*., 1988), 1-pentadecanol (Marongiu *et al*., 2003; Laurence *et al*., 1999; Dauben, 1948; Ruhoff and Reid, 1933; Kao and Shao-Yuan, 1922) were isolated (Fig. 1).

MATERIALS AND METHODS

Plant Material

Fresh aerial parts of *Schinus terebinthifolius* RADDI (Anacardiaceae) were collected in June, 2004 from Suez Canal University garden, Ismailia, Egypt. The identity was established by Prof. Dr. Hamdy K. Atta-Alla, Prof. of Floriculture and Medicinal plants, Department of Horticulture, Faculty of Agriculture, Suez Canal University. A voucher specimen (Number AMYM-1001) has been deposited in the Herbarium of Botany Department, Faculty of Science, Suez Canal University, Ismailia, Egypt.

General Methods

Melting points were determined on Büchi 535 melting point apparatus and are uncorrected. IR spectra were recorded on Brnker FTIR vector 22 spectrophotometer, in KBr disks. UV spectra were obtained on Hitachi UV 3200 spectrophotometer. EIMS (ionization voltage 70 ev) was

measured on a Varian MAT 311/A mass spectrometer and HREIMS were taken by MS JEOL-MS route, JMS-600H, Agilent 6890N. Fab-JEOL. JMS-HX 110 Mass spectrometer, glycerol was used as the matrix. 1D and 2D NMR spectra were run on Bruker AMX 400 and AMX 500 MHZ NMR spectrometers. The chemical shifts are given in ppm (δ), relative to TMS as internal standard and coupling constants are in Hz.

Single Crystal Structure Determination

A block shaped yellowish crystal of compound 4 $C_8H_9NO_2$: Mr 151.4 with dimension 0.61×0.31×0.24 mm was selected for X-ray diffraction studies.

Crystal data for the structure of 4 is presented in Table 1.

Intensity data of compound 4 was collected on a Bruker Smart CCD 1-K area-detector diffractometer using Mo-Kα radiation (λ = 0.7107 Å) (Siemens, SMART and SAINT, 1996). Data reductions were performed using SAINT. The structure was solved by direct methods (Altomare *et al*., 1993) and refined by full-matrix least squares on F^2 using the SHELXTL-PC package (Sheldrick, 1997). The intensity data within the θ range 2.52-24.99 were collected at 293 (2) K. The figure was plotted with the aid of ORTEP (Jhnson, 1976). Crystallographic data for compound 4 has been deposited to Cambridge Crystallographic Data Center (CCDC 610583), 12 Union Road, Cambridge, CB/EZ, UK (Fax: 44-1223-336-033, e-mail: deposit@ccdc.cam.ac.uk).

Column chromatography was carried out on silica gel (70-230 mesh, Merck). TLC was performed on Merck precoated silica gel 60 F_{254} aluminium foil plates and detection was achieved by UV light (254 um), *p*-dimethylaminobenzaldehyde and iodine solution.

Extraction, Isolation and Characterization

Air-dried and powder aerial parts (5.0 kg) of the plant were macerated with ethanol 80% at room temperature till exhaustion. The resulting alcoholic extract was concentrated in vacuo to obtain a crude residue (3.0 kg). A part of this residue (1.0 kg) was dissolved in distilled water (600 mL) and defatted with *n*-hexane then acidified with glacial acetic acid to pH 3-4. The acidic solution was exhaustively extracted 5 times with $CHCl_3$ (5×500 mL) to yield the acidic chloroform extract (132.2 g). The aqueous solution was basified with 10% NH_4OH (pH 8-9) and re-extracted with

Table 1: Crystal data, details of the data collection and structure analysis of compound 4

Compound	4
Crystal color, shape	Yellow, block
Crystal size [mm]	0.61×0.31×0.24
Empirical formula	$C_{16}H_{18}N_2O_4$
Chemical formula	$C_{16}H_{18}N_2O_4$
Formula weight	302.32
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions [pm], angles [°]	a = 509.68(5), b = 951.77(10) c = 1538.91(16), α = β = γ = 90
Volume [10^6 pm^3]	746.52(13)
Z	4
Density (calculated) [g cm^{-3}]	1.345
Absorption coefficient [mm^{-1}]	0.098
F(000)	320
Goodness-of-fit on F^2	1.125
Collected reflections	3757
θ range for data collection [°]	2.52-24.99
Completeness to maximum θ [%]	99.9
Index ranges	-5 ≤ h ≤ 6,-11 ≤ k ≤ 11,-18 ≤ l ≤ 15
Final R indices R1/*w*R2 [I≥2 σ (I)]	0.0629/0.1939
R indices R1/*w*R2 (all data)	0.0633/0.1948
Max./min. e-density [10^{-6} e. * pm^{-3}]	0.399/-0.380

chloroform to yield the basic chloroform extract (10.17 g) as a brown gummy residue, which represented 1.02% total crude alkaloids of the dry plant material. A portion of the basic chloroform extract (9 g) was chromatographied over 350 g of silica gel and eluted with dichloromethane-methanol with the gradient polarity (0-40%). A total of 155 fractions ca. 150 mL each were collected and combined on the basis of TLC analysis leading to 15 series (A-O). Further purification of these series was achieved by column chromatography and preparative thin layer chromatography. Series A (211 mg), series B (503 mg), series C (240 mg) and series D (504 mg) obtained with 100% dichloromethane, upon examination by TLC (CH_2Cl_2-MeOH, 97: 3+1 drop of diethyl amine) contained a complex mixture with small amounts, were not investigated. Series E (1500 mg) obtained with CH_2Cl_2-MeOH (98:2) was purified successively on a silica gel column chromatography with CH_2Cl_2 and increasing the polarity with MeOH (up to 5%) to furnish compound 1 (869.3 mg).

Series F (728.9 mg) obtained with CH_2Cl_2-MeOH (96:4) was rechromatographed on a silica gel column chromatography eluting with CH_2Cl_2 and increasing the polarity with MeOH to yield 253.2 mg of compound 2 and a mixture of two compounds, which was subjected to further purification with preparative thin layer chromatography to afford 95.2 mg of compound 3 and 102.3 mg of compound 7.

Series G (2192.3 mg) obtained with CH_2Cl_2-MeOH (94:6) was also subjected to column chromatography over silica gel with CH_2Cl_2 and increasing the polarity with MeOH to afford 569.2 mg of compound 4 and a mixture of two compounds which subjected to further purification using PTLC to yield 456.2 mg of compound 5 and more amount from compound 1 (34.2 mg).

Series H (552.2 mg), obtained with CH_2Cl_2-MeOH (90:10) was separated using the same conditions as above, afforded more amount from compound 1 (210.3 mg) and a mixture of two compounds which applied on PTLC for further purification to yield more amount of compound 5 (23.4 mg).

Series I (2032.2 mg), obtained with CH_2Cl_2-MeOH (88:12) was applied to a silica gel column chromatography and eluted with CH_2Cl_2: MeOH of increasing polarity, to furnish 1343.7 mg of compound 6.

(4-aminophenyl) acetic acid (4). R_f; (0.35, CH_2Cl_2:MeOH, 95%, 1 drop of diethyl amine), yellow needle crystals in dichloromethane-methanol; m.p. 170-172°C; UV λ_{max} nm (MeOH) (log ε): 202 (4.26), 211 (3.99), 251 (3.08), 226 (4.17), 278 (3.60), 366 (2.40), 390 (2.52); IR bands (KBr) ν_{max}: 3394, 3217, 2925, 2862, 2673, 2574, 2492, 1895, 1660, 1606, 1512, 1446, 1294, 1232, 1178, 1109, 1026, 864, 798, 682, 657, 567, 526 cm^{-1}. ^1H NMR (400 MHZ, pyridine-d_5): δ 11.33 (1H, *s*, OH-10), 7.74, 7.88 (1H each, 2x *br s*, NH_2), 7.43 (2H, *d*, *J* 8.39, H-3, H-5), 7.13 (2H, *d*, *J* 8.36, H-2, H-6), 3.74 (2H, *s*, H-8); ^{13}C NMR (125 MHZ, CD_3OD): 177.6 (C-9), 157.4 (C-1), 127.6 (C-4), 131.1 (C-3, C-5), 116.3 (C-2, C-6), 42.6 (C-8); HR EIMS *m/z*: 151.0637 (calcd. for $C_8H_9O_2N$, 151.0633); EIMS *m/z* (rel. Int.): 151 (28), 107 (100), 90 (5), 77.0 (26), 55 (4), 51 (15).

2-phenylacetamide (1). R_f; (0.60, CH_2Cl_2:MeOH, 95%, 1 drop of diethyl amine), Beige crystals in dichloromethane-methanol; m.p. 156-158°C; UV λ_{max} nm (MeOH) (log ε): 206 (3.52), 241 (2.32), 258 (2.46), 330 (1.84), 351 (2.24), 366 (2.08), 390 (2.40); IR bands (KBr) ν_{max}: 3357, 3178, 2925, 2856, 2806, 1949, 1639, 1450, 1415, 1286, 1180, 1130, 1072, 1029, 910, 871, 746, 698, 584, 536, 474 cm^{-1}. ^1H NMR (500 MHZ, $CDCl_3$): δ 5.50, 5.88 (1H each, 2x *br s*, NH_2), 7.24-7.34 (5H, Ar-H), 3.55 (2H, *s*, H-7); ^{13}C NMR (125 MHZ, CD_3OD): δ 176.96 (C-8), 130.14 (C-2, C-6), 129.56 (C-3, C-5), 127.8 (C-4), 136.90 (C-1), 43.42 (C-7); HR EIMS *m/z*: 135.0672 (calcd. for C_8H_9ON, 135.0684); EIMS *m/z* (rel. Int.): 135 (20), 107 (4), 92 (100), 91 (96), 77 (2).

Ethyl gallate (2). R_f; (0.52, CH_2Cl_2:MeOH, 95%, 1 drop of diethyl amine), pink needle crystals in dichloromethane-methanol; reacted positively with $FeCl_3$ reagent and give green color; m.p.146-148°C; UV λ_{max} nm (MeOH) (log ε): 218 (4.59), 241 (3.50), 275 (4.20), 340 (2.38), 341 (2.53), 365 (2.33), 389 (2.62); IR bands (KBr) ν_{max}: 3454, 3305, 2968, 2927, 2857, 1705, 1618, 1533,

1455, 1409, 1317, 1254, 1197, 1098, 1035, 967, 867, 762, 609 cm^{-1}. ^1H NMR (500 MHZ, CD_3OD): δ 7.03 (2H, *s*, H-2, H-6), 4.25 (2H, *q*,-CH_2), 1.33 (3H, *t*,-CH_3), 11.58 (3OH, *s*, OH-3, OH-4, OH-5, in pyridine-d_5); ^{13}C NMR (125 MHZ, CD_3OD): δ168.5 (C-7), 146.4 (C-3, C-5), 139.7 (C-4), 121.8 (C-1), 110.0 (C-2, C-6), 61.6 (C-8), 14.6 (C-9); HR EIMS m/z: 198.0516 (calcd. for $C_9H_{10}O_5$, 198.0528); EIMS m/z (rel. Int.): 198 (86), 183 (10), 169 (3), 153 (100), 125 (28), 107 (5), 79 (11).

Benzamide (3). R_f; (0.64, CH_2Cl_2:MeOH, 90%, 1 drop of diethyl amine), white powder; m.p. 131-133°C; UV λ_{max} nm (MeOH) (log ε): 204 (3.93), 271 (3.69), 235 (3.50), 738 (3.10), 746 (3.15), 818 (3.04), 823 (3.07), 839 (3.04), 843 (3.06); IR bands (KBr) ν_{max}: 3365, 3175, 3029, 2922, 2853, 1952, 1660, 1637, 1605, 1601, 1495, 1451, 1415, 1286, 1286, 1246, 1183, 1117, 1074, 968, 936, 863, 747, 700, 584, 532, 475 cm^{-1}. ^1H NMR (500 MHZ, CD_3OD): δ7.44 (2H, *dd*, *J* 7.62Hz, H-3, H-5), 7.85 (2H, *d*, *J* 7.44Hz, H-2, H-6), δ7.53 (2H, *dd*, *J* 7.43Hz, H-4), 8.36, 8.34 (1H each, 2x *br s*, NH_2, in pyridine-d_5); ^{13}C NMR (125 MHZ, CD_3OD): δ134.98 (C-1), δ132.92 (C-4), δ128.85 (C-3, C-5), δ128.64 (C-2, C-6), δ172.43 (C-7); HR EIMS *m/z*: 121.052764 (calcd. for C_7H_7ON, 121.05275); FABMS (+ve) $[M+1]^+$: 122; EIMS *m/z* (rel. Int.): 121 (62), 105 (88), 77 (100).

3-(4-aminophenyl)prop-2-enoic acid (5). R_f; (0.33, CH_2Cl_2:MeOH, 95%, 1 drop of diethyl amine), white crystals in dichloromethane-methanol; m.p.153-155°C; UV λ_{max} nm (MeOH) (log ε): 209 (4.22), 226 (4.37), 249 (3.42), 285 (4.11), 303 (4.04), 308 (4.05), 368 (2.43), 389 (2.50); IR bands (KBr) ν_{max}: 3396, 3220, 2922, 1899, 1659, 1609, 1601, 1514, 1411, 1291, 1232, 1176, 1108, 989, 943, 890, 859, 826, 798, 682, 566, 528, 448 cm^{-1}. ^1H NMR (300 MHZ, CD_3OD): δ 7.40 (2H, *d*, *J* 8.28, H-3, H-5), 6.78 (2H, *d*, *J* 8.29, H-2, H-6), 7.46 (2H, *d*, *J* 15.99, H-8), 6.43 (2H, *d*, *J* 15.76, H-9), 11.41 (1H, *s*, OH-10 in pyridine-d_5), 7.99, 7.88 (1H each, 2x *br s*, NH , in pyridine-d); ^{13}C NMR (75 MHZ, CD_3OD): 171.63 (C-10), 160.67 (C-1), 127.58 (C-4), 130.68 (C-3, C-5), 116.74 (C-2, C-6), 142.93 (C-8), 117.84 (C-9); HR EIMS *m/z*: 163.0627 (calcd. for $C_9H_9O_2N$, 163.0633); EIMS *m/z* (rel. Int.): 163 (32), 162 (19), 120 (9), 119 (28), 107 (100), 94 (4), 93 (3), 77 (53).

1-pentadecanol (6). white needle crystals in dichloromethane-methanol; m.p. 44-46°C; IR bands (KBr) ν_{max}: 3453, 2920, 2851, 1740, 1656, 1601, 1469, 1381, 1249, 1219, 1083, 1018, 995, 972, 916, 833, 765, 722, 668, 634, 560, 471 cm^{-1}. ^1H NMR (500 MHZ, CD_3OD): δ 0.88 (3H, *t*, *J* 6.86,-CH_3), 1.28-1.36 (2H each, *m*, 12-CH_2), 1.64 (2H, *p*, *J* 6.98, CH_2-2), 3.97 (2H, t, *J* 6.59, CH_2-1); ^{13}C NMR (125 MHZ, CD_3OD): δ 69.15 (C-1), 33.05 (C-2), 30.37-30.76 (C-4-13), 26.89 (C-3), 23.70 (C-14), 14.39 (C-15); FABMS (+ve) $[M+1]^+$: 229; (calcd. for $C_{15}H_{32}O$, 228.245303); EIMS m/z (rel. Int.): 196 (9.1), 168 (39.1), 140 (16.6), 126 (10.1), 112 (17.2). 98 (25.8), 84 (38.8), 70 (49.8), 56 (53).

Cinnamic acid (7). White crystals in dichloromethane-methanol; m.p. 132-134°C; ^1H NMR (500 MHZ, CD_3OD): δ7.24 (2H, *dd*, *J* 8.32Hz, H-3, H-5), δ7.55 (2H, *d*, *J* 7.84Hz, H-2, H-6), δ7.37 (2H, *dd*, *J* 7.47Hz, H-4), δ7.54 (2H, *d*, *J* 16.09Hz, H-7), δ6.63 (2H, *d*, *J* 15.80Hz, H-8); ^{13}C NMR (75 MHZ, CD_3OD): δ164.98 (C-9), δ136.20 (C-1), δ130.95 (C-4), δ129.57 (C-3, C-5), δ128.91 (C-2, C-6), δ142.73 (C-7), 121.43 (C-8); HR EIMS *m/z*: 148.05243 (calcd. for $C_9H_8O_2$, 148.0524262); FABMS (+ve) $[M+1]^+$: 149; EIMS *m/z* (rel. Int.): 148 (9), 147 (61), 146 (100), 104 (12), 103 (96), 91 (93), 90 (6), 77 (75).

Antifungal Assay

The microorganisms used in the antifungal assays *Trichphton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsorum canis*, *Fusarium solani* and *Candida glabrata* have been maintained from Microbiology department, Karachi university, Karachi, Pakistan. Stock solutions of each sample were freshly prepared in 1 mL dimethylsulfoxide (DMSO). These solutions were diluted into sterile molten Sabouraud dextrose agar (SDA) medium to reach a final concentration of 200 μg mL^{-1} separately. Test tubes were kept at room temperature for solidification. Medium containing DMSO was used as negative control. Fungal cultures were cut to 4×4 mm from 1 week grown plates and then inoculated onto the slant. After an incubation period of 7-10 days at 29°C, tubes were examined for

the growth inhibition. Growth on the media containing compound was determined by measuring the linear growth (mm) of fungal culture (Atta-ur-Rahman *et al*., 2001). Growth inhibition (%) was calculated with reference to the negative control.

Antioxidant Assay (DPPH (1, 1-diphenyl-2-picryl Hydrazyl) Free Radical Scavenging Activity)

The reaction mixture containing 5 µL of test sample (1 mm in DMSO) and 95 µL of DPPH (Sigma, 300 µm) in ethanol the reaction mixture was taken in a 96-well micro titer plate (Molecular Devices, USA) and incubated at 37°C for 30 min. The absorbance was measured at 515 nm. Percent radical scavenging activity determined by comparison with a DMSO containing control. IC_{50} values represent concentration of compounds to scavenge 50% of DPPH radicals. BHA (3-t-Butyl-4-hydroxyanisole) was used as a positive control. All the chemicals used were of analytical grade (Sigma, USA) (Gulcin *et al*., 2004).

Anti-Alzheimer`s Assay

***In vitro* Cholinesterase Inhibition Assay**

Electric-eel acetylcholinesterase (EC 3.1.1.7), horse-serum butyrylcholinesterse (E.C 3.1.1.8), acetylthiocholine iodide, butyrylthiocholine chloride, 5,5′-dithiobis [2-nitrobenzoic acid] (DTNB) and galanthamine were purchased from Sigma (St.Louis, MO, USA). All other chemicals were analytical grade. Acetylcholinesterase and butyrylcholinesterase inhibiting activities were measured by the spectrophotometric method developed by Ellman *et al*. (1961). Acetylthiocholine iodide and butyrylthiocholine chloride were used as substrates to assay acetylcholinesterase and butyrylcholinesterase, respectively. The reaction mixture contained 150 µL of (100 mM) sodinm phosphate buffer (pH 8), 10 µL of DTNB, 10 µL of test-compound solution and 20 µL of acetylcholinesterase or butyrylcholinesterase solution, which were mixed and incubated for 15 min (25°C). The reaction was then initiated by the addition of 10 µL acetylthiocholine or butyrylthiocholine, respectively. The hydrolysis of acetylthiocholine and butyrylthiocholine were monitored by the formation of yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine and butyrylthiocholine, respectively at a wavelength of 412 nm (15 min). Test extract and the positive control (Galanthamine and Eserine) were dissolved in EtOH. All the reactions were performed in triplicate in 96-well micro-plate in *SpectraMax 340* (Molecular Devices, USA). The percentage (%) inhibition was calculated as follows (E-S)/E x 100, where E is the activity of the enzyme without test compound and S is the activity of enzyme with test compound (Tougu, 2001).

Determination of IC_{50} Values

The concentrations of test compounds that inhibited the hydrolysis of substrates (acetylthiocholine and butyrylthiocholine) by 50% (IC_{50}) were determined by monitoring the effect of increasing concentrations of these compounds in the assays on the inhibition values. The IC_{50} values were then calculated using the EZ-Fit Enzyme Kinetics program (Perrella Scientific Inc., Amherst, USA).

Anti-leishmanial Assay

Leishmania major prmastigotes (DESTO), cultivated in bulk were aseptically be sedimented down at 3000 rpm, counted with the help of improved Neubavr chamber under the microscope and diluted with the fresh medium to a final concentration of 1×10^6 parasites/mL. The compound to be checked were dissolved to a final concentration of 1.0 mg in 0.1 mL of PBS (Phosphate Buffered Saline, pH 7.4 containing 0.5% MeOH, 0.5% DMSO). In 96-well microtiter plate, 180 µL of the parasite culture (1.0×106 parasites/mL) was added in difference wells. Twenty microliter of the experimental

compound was added in culture and serially diluted so that minimum concentration of the compound is 0.1 μg mL^{-1}. Ten microliter of PBS was added as negative control while glucantime, amphotericin B, pentaamidine and ampicilline to a final concentration of 0.1 mg mL^{-1} was added separately as positive control. The plate was incubated between 21-22°C in dark for 2 h. The culture was examined microscopically on an improved neubaver chamber and IC_{50} values of compounds possessing antileishmanial activity were calculated (Habtemariam, 2003). All assays were run in duplicate.

RESULTS AND DISCUSSION

The basic chloroform extract of the finely powder aerial part of *Schinus terebisifolius* was subjected to column chromatography. The fractions obtained were subjected to different sub-columns and preparative thin layer chromatography, afforded one new naturally occurring compound, synthetically known named (4-aminophenyl) acetic acid, along with the known 2-phenylacetamide, 1-pentadecanol, 3-(4-aminophenyl) prop-2-enoic acid, (*E*), ethyl gallate cinnamic acid and benzamide. The known compounds were identified by comparison of their spectral analysis data with the published ones (Schwartz *et al*., 1987; Giridhar *et al*., 2003; Manley and Bilodeau, 2004; Firouzabadi *et al*., 1998; Guranda *et al*., 2001; Peng *et al*., 2003; Aleksi *et al*., 2001; Ono *et al*., 1999; Shingo *et al*., 2003; Marco *et al*., 1978; Ripperger *et al*., 1981; Persinos *et al*., 1967; Douglas *et al*.,1997; Buller *et al*., 1992; Lord *et al*., 1973; Cook, 1989; Mehta *et al*., 1988; Marongiu *et al*., 2003; Laurence *et al*., 1999; Dauben, 1948; Ruhoff and Reid, 1933; Kao and Shao-Yuan, 1922).

(4-aminophenyl) acetic acid was obtained as yellow needle crystals, m.p. 170-172°C. Its showed violet color under UV-light (λ 254 um) and reacted positively with *p*-dimethylaminobenzaldehyde reagent. The molecular formula was determined as $C_8H_9O_2N$ by HR EIMS [M^+] m/z: 151.0637, in conjunction with the NMR spectra. The IR spectrum showed hydroxyl group (3394 cm^{-1}), amino group (2673-2492 cm^{-1}), carbonyl group (1660 cm^{-1}). In ^{1}H-NMR spectrum we observed a singlet of two protons at 3.74 corresponding for a methylene group and in the region of aromatic proton, a typical AA`BB` system at 7.43 and 7.13 (2H each, *d*, *J* 8.39) can also been observed. The mass spectrum showed a base peak at 107 corresponding to the loss of one carboxylic group. This was confirmed in the ^{1}H-NMR spectrum, which displayed a singlet of one hydroxyl group at δ11.33. In addition, the ^{1}H-NMR spectrum showed two broad singlet of one proton each at δ7.74 and 7.88 due to the amine group. This group was deduced to be in Para position with the methylene group according to the HMBC spectrum in which we observed the ^3J correlation between the proton signal at δ 3.74 and C-2. On the other hand, the signal at δ7.88 was assigned for C-3. The assigument of all the carbons was possible from HMBC and COSY correlation experiment.

X-ray Crystal Structure Analysis of (4)

X-ray structure analysis was possible for compound 4. This compound crystallizes from dichloromethane-methanol at room temperature as yellow block in the orthorhombic space group $P2_12_12_1$, with a = 509.68(5), b = 951.77(10), c = 1538.91(16) pm, α = β = γ = 90°, V = 746.52(13) $\times 10^6$ pm^3 and Z = 4. The crystallographic data are listed in Table 1. The solid-state structure of 4 is shown in Fig. 2.

In the crystal lattice of compound 4, one molecule interacts with four nearest neighbors, which results in a net motif, as depicted in Fig. 2b. It is interesting to note that the intermolecular aryl groups are directly stacked over one another for every two molecules. The dotted lines in Fig. 2b represent distances of 296.5(5), 276.2(4) and 292.8(4) pm for the N1….O2, N1….O1 and O2….O1 interactions, respectively (Table 2).

From the above mention the structure of compound 4 was deduced to (4-aminophenyl) acetic acid. This compound was already synthesized by Schwartz *et al*. (1987), but it is the first time to isolate from any plant, so it is new naturally compound.

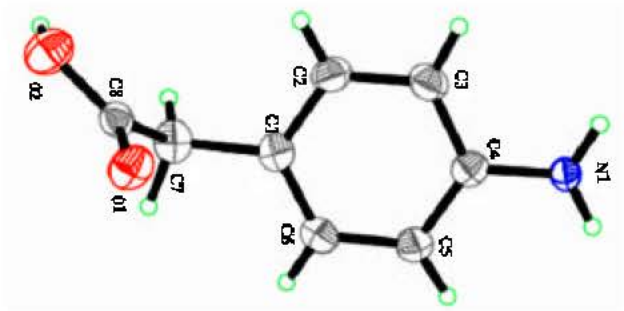

Fig. 2a: ORTEP drawing (50% probability level) of compound 4. Selected bond lengths [pm]: N(1)-C(4) 136.0(5), C(1)-C(7) 151.3(6), C(7)-C(8) 151.9(6); O(1)-C(8) 125.3(5); O(2)-C(8) 131.6(5); selected bond angels [°]: N(1)-C(4)-C(3) 118.3(3), N(1)-C(4)-C(5) 122.2(4), C(2)-C(1)-C(7) 120.2(4); C(6)-C(1)-C(7) 121.9(4); C(1)-C(7)-C(8) 112.7(3); O(1)-C(8)-C(7) 120.9(4); O(1)-C(8)-O(2) 121.7(4); selected torsion angles [°]: C(2)-C(3)-C(4)-N(1) 179.3(4), N(1)-C(4)-C(5)-C(6)-179.6(4), C(2)-C(1)-C(7)-C(8)-68.6(5), C(6)-C(1)-C(7)-C(8) 111.7(4)

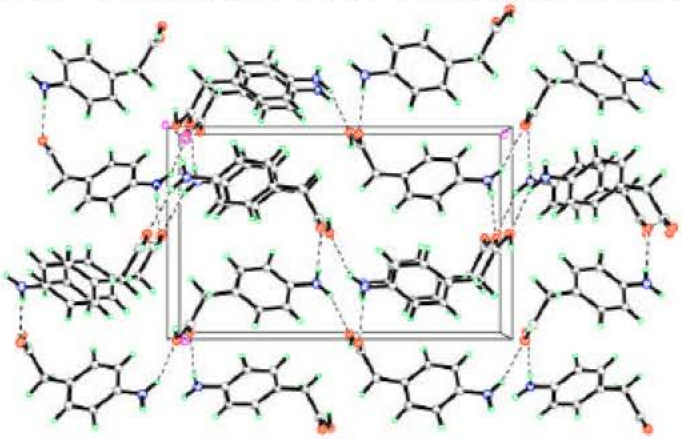

Fig. 2b: The crystal packing of compound 4 along a-axis, showing H-bonding in dashed-lines

Total alcoholic extract of *Schinus terebinthifolius* showed significant activity against *Trichophyton logifusus* and *Candida albicans*. Compound 1 and 2 showed moderated activity against *Trichphyton longifusus* and *Microsporum canis*, compound 4 showed good activity against *Microsporum canis* and moderate activity against *Trichphyton longifusus*, compound 5 showed moderate activity against *Trichophyton logifusus* and *Microsporum canis* and compound 6 showed good activity against *Trichophyton logifusus*, *Microsporum canis and Aspergillus flavus,* while compounds 3 and 7 were not investigated because they have small amounts (Table 3).

On the other hand, total alcoholic extract of *Schinus terebinthifolius* showed very significant activity (92.8%) against DPPH Radical at 200 μg mL^{-1} (Table 3) and the isolated compound 2 showed very significant activity (96.1%) at 1 mM; compound 4 displayed moderate activity (50%) and the other compounds showed low activity. Compound 1 showed low antibacterial activity against *Shigella flexnari*, while the other compounds showed non-significant antibacterial activities (Table 4).

The total alcoholic extract gave significant inhibition for alzheimer`s as shown in Table 5, good antileishmanicidal activity. On the other hand, it gave low antibacterial activity against *Pseudomonas auruginosa* and *Salamonella typhi*, non-significant inhibition against insecticidal activity, non-significant phytotoxicity and non-significant cytotoxicity.

Table 2: Intermolecular hydrogen bond distances [pm] and angles [°] of the participating moieties of 4

D-H...A	D-H	H...A	D...A	< (DHA)
N(1)-H(1A)...O(2)[ii]	0.8589	2.2811	296.5(5)	136.7
N(1)-H(1B)...O(1)[iii]	0.8603	2.0469	276.2(4)	139.9
O(2)-H(1O2)...O(1)[I]	1.15(6)	1.94(7)	292.8(4)	141.0

Symmetry codes (I)-1+x,y,z (ii)-½-x,-y,1/2+z (iii)-x,1/2+y,3/2-z

Table 3: *In vitro* antifungal bioassay (agar tube dilution protocol)

Name of the fungus	% Inhibition						Standard drugs	MIC of standard Drug (μg mL^{-1})
	Total Alc. extract	C-1	C-2	C-4	C-5	C-6		
Trichophyton longifusis	83	50	50	60	50	65	Miconazole	70
Candida albicans	90	0	0	0	0	0	Miconazole	110.8
Aspergillus flavus	0	0	0	0	0	70	Amphotericin	20
Microsporum canis	80	45	55	70	45	70	Miconazole	98.4
Fusarium solani	65	0	0	0	20	40	Miconazole	73.25
Candida glbarata	0	0	0	0	0	0	Miconazole	110.8

MIC: Minimum Inhibitory Concentration, C: Compound

Table 4: DPPH (1, 1-Diphenyl-2-picryl hydrazyl) free radical scavenging activity

Code of sample	[c]IC_{50} (mM) ± [b]SEM	DPPH radical scavenging activity
Total alcoholic Extract	-	92.8 (at 200 μg mL^{-1})
[a]C-1	-	10.0 (at 1 mM)
C-2	0.1418±0.003	96.1 (at 1 mM)
C-3	-	-
C-4	-	50.0 (at 1 mM)
C-5	-	40.1 (at 1 mM)
C-6	-	-
Standard		
3-*t*-Butyl-4-Hydroxy anisole	0.044 ± 0.001	92.1 (at 1mM)

[a]C : Compound, [b]SEM: Standard error of mean, [c]IC_{50}: The concentration of sample required to inhibit 50% of DPPH radical

Table 5: Bioassay results of the total alcoholic extract of *Schinus terebinthifolius*

Type of bioassay	% inhibition	Comment
Enzyme inhibition studies		
Acetyl choline esterase	75.4 (1 mg mL^{-1})	Significant inhibition for Alzheimer`s
Butyl choline esterase	100	
Antiinflammatory activity	5.39	Non-significant inhibition
Antileishmanial activity	IC_{50} 76.67 μg mL^{-1}	Good Leishmanicidal activity
Antiinsecticidal activity	20	Non-significant activity against *Rhyzopertha dominica*
Antibacterial activity	15 mm (*Pseudomonas auruginosa* and *Salamonella typhi*)	Low antibacterial activity against *Pseudomonas anruginosa* and *Salamonella typhi*
Phytotoxic activity	80 at the highest concentration (1000 μg mL^{-1})	Non-significant phytotoxicity
Brine-shrimp activity	No positive cytotoxicity	Non-significant cytotoxicity

ACKNOWLEDGMENTS

The first author is grateful to HEJ Institute of chemistry, Karachi University, Karachi, Pakistan for the Fellowship it has provided her with to undertake this work. The authors thank the bioassays lab members for bioassay analysis.

REFERENCES

Aleksi, E.S., V.Y. Dmitri, J.M. Niko and V.P. Gelii, 2001. Preparation of monofunctional and phosphorescent palladium (II) and platinum (II) coproporphyrin labeling reagents. J. Porphyrins Phthalocyanines, 5: 735.

Altomare, A., M. Cascarano, C. Giacovazzo and A. Guagliardi, 1993. Completion and refinement of crystal structures with SIR92. J. Applied Cryst., 26: 343-350.

Atta-ur-Rahman, M.I. Choudhary and W. Thompson, 2001. A Manual of Bioassay Techniques for Natural Products Research, Harwood Academic Press Inc., London.

Buller, B.T., G. Silvey, D.M. Houston, D.R. Borcherding, V.L.Vaughn, A.T. McPhail, D.M. Radzik, H. Wynberg, W. ten Hoeve and E. van Echten, 1992. The resolution, isolation and pharmacological characterization of the enantiomers of a benzamide containing a chiral sulfoxide. Chirality, 4: 155-162.

Campello, J.P. and A.J. Marsaioli, 1974. Triterpenes of *Schinus terebinthifolius*. Phytochem. Reports, 13: 659-660.

Campello, J.P. and A.J. Marsaioli, 1975. Terebenthifolic acid and bauerenone: New Triterpenoid Ketones from *Schinus terebinthifolius*. Phytochemistry, 14: 2300-2302.

Cook, I.B., 1989. Caveat regarding the use of substituent parameters in statistical analyses of molecular properties. II. Case Study: ^{13}NMR of 2-Substituted Pyridines and Monosubstituted Benzenes Aust. J. Chem., 42: 1493-1518.

Dauben, W.G., 1948. The synthesis of palmitic acid and tripalmitin labeled with carbon fourteen. J. Am. Chem. Soc., 70: 1376-1378.

Douglas, E.D., J.L. Leslie, L.O. John, A.S. Larry, W.C. Monte, N.B. Fadia and W.B. Steven, 1997. Isolation and structure elucidation of the major degradation products of cefaclor in the solid state. J. Pharm. Sci., 86: 540-549.

Ellman, G.L., K.D. Courtney, V. Jr. Andres and R.M. Feather-stone, 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7: 88-95.

Firouzabadi, H., M. Adibi and B. Zeynizadeh, 1998. Modified borohydride agents; Efficient reduction of azides with (1,4-Diazabicyclo [2.2.2] Octane) (Tetrahydroborato) zinc complex [$Zn(BH_4)_2$(dabco)] and Methyl-triphenylphosphonium Tetrahydroborate [$MePh_3PBH_4$]). Synth. Commun., 28: 1257-1273.

Giridhar, P., R.D. Vijava, B.O. Reddy, T. Rajasekaran and G.A. Ravishankar, 2003. Influence of Phenylacetic acid on clonal propagation of *decalepis hamiltonii* wight and arn: An endangered shrub. *In vitro* Cellular and Development Biology-Plant, 39: 463-467.

Gulcin, O., K. Irfan, M. Oktay and M.B. Emin, 2004. Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica L.*). J. Ethnopharmacol., 90: 205-215.

Guranda, D.T., L.M. van Langen, F. van Rantwijk, R.A. Sheldon and V.K. Švedas, 2001. Highly efficient and enantioselective enzymatic acylation of amines in an aqueous medium. Tetrahedron: Asymmetry, Jaargang, 12: 1645-1650.

Habtemariam, S., 2003. *In vitro* antileshmanial effects of antibacterial diterpenes from two Ethiopian *Premna species: P. schimperi* and *P. oligotricha*. BMC Pharmacol., 3: 6.

Hayashi, T., K. Nagayama,, M. Arisawa, M. Shimizu and Sh. Suzuki *et al.*, 1990. Pentagalloyglucose, a xantine oxidase inhibitor from a paraguayan crude drug, Molle-I (*Schinus terebinthifolius*). J. Nat. Prod., 52: 210-211.

Jain, M.K., Yu. Bao-Zhu, J.M. Rogers, A.E. Smith, E.T. Boger, R.L. Ostrander and A.L. Rheingold, 1995. Two structurally related riterpenoids 1 and 2 from pink peppercorn (berries of *Schinus terebinthifolium*). Phytochemistry, 39: 537-547.

Jhnson, C.K., 1976. ORTEPII. Report ORNL-5138. Oak Ridge National Laboratory, Tennessee, USA.

Kaistha, K.K. and L.B. Kier, 1962a. Structural studies on terebinthone from *Schinus terebinthifolius*. J. Pharm. Sci., 51: 245-248.

Kaistha, K.K. and L.B. Kier, 1962b. Structural studies on triterpenes of *Schinus terebinthefolius*. J. Pharm. Sci., 51: 1136.

Kao, H. and M. A. Shao-Yuan, 1922. The preparation of benzamide. Notes and Goebel. J. Am. Chem. Soc., 44: 2286.

Laurence, M., S. Alain, B. Antony, Y. Bin, E.S. Ruth and R. Ulrich, 1999. Glycerol is a suberin monomer. New experimental evidence for an old hypothesis. Plant Physiol., 119: 1137-1146.

Lord, G.H., B.J. Millard and J. Memel, 1973. High resolution mass spectrometry. Part X. Loss of water from the molecular ions of aromatic amides. J. Chem. Soc., Perkin Trans., 1: 572-574.

Manley, P.J. and M.T. Bilodeau, 2004. A new synthesis of naphthyridinones and quinolinones: Palladium-catalyzed amidation of *O*-carbonyl-substituted aryl halides. Org. Lett.Commun., 6: 2433-2435.

Marco, J.A., J.S. Parareda, E. Seoane, B. Abarca and J.M. Sendra, 1978. Waxes, triterpenes and free acids in *Anthyllis sericea*. Phytochemistry, 17: 1438.

Marongiu, B., S. Porcedda, A. Caredda, B. De Gioannis, L. Vargiu and P.La Colla, 2003. Extraction of *Juniperus oxycedrus* sp. *oxycedrus* essential oil by supercritical carbon dioxide: Influence of some process parameters and biological activity. Flavour and Fragrance J., 18: 390-397.

Mehta, B.K., Sh. Savita and D. Avinash, 1988. 4-Ethylgallic acid from two mimosa species. Phytochemistry, 27: 3004-3005.

Mitchell, J.D., 1990. The poisonous *Anacardiaceae genera* of the world. Adv. Econ. Bot., 8: 103-129.

Morton, J.F., 1978. Brazilian pepper-its impact on people, animals and the environment. Econ. Bot., 32: 354-360.

Ono, S., T. Yoshida, K. Maeda, K. Kosaka, Y. Inoue, T. Imada, C. Fukaya and N. Nakamura, 1999. Preparation and pharmacological evaluation of novel glycoprotein (Gp) IIb/IIIa antagonists. 2. Condensed heterocyclic derivatives. Chem. Pharm. Bull. (Tokyo). 47: 1694-1712.

Peng, J., Hu. Jin-Feng, A.B. Kazi, Ze. Li., M. Avery and O. Peraud *et al*., 2003. Manadomanzamines a and b: A novel antimycobacterial manzamine ring system from an indonesian sponge *Petrosiidae* sp. J. Am. Chem. Soc., 125: 13382-13386.

Persinos, G.J., M.W. Quimby, A.R. Mott, N.R. Farnsworth, D.J. Abraham, H.H. Fong and R.N. Blomster, 1967. Studies on Nigerian Plants III. Biological and phytochemical screening of *Lophira lanceolata* and the isolation of benzamide. Planta Med., 15: 361-365.

Ripperger, H., M. Diaz and K. Schreiber, 1981. Cinnamoyl derivatives from *Cinnamomum-triplinervis*. Phytochemistry, 20: 1453-1454.

Ronald, C., 1999. Leaf-litter inhabitants of a Brazilian pepper stand in Everglades National Park. Florida Entomol., 82: 388-403.

Ruhoff, J.R. and E.E. Reid, 1933. A group of isomeric esters. J. Am. Chem. Soc., 55: 3825-3828.

Sarita Varma, M., 2002. Brazilian pepper masquerades as the indian variety. Relishing the Flavor. Economist Technol. Quart., 22: 28-29.

Schwartz, L.M., R.I. Gelb, J. Mumford-Zisk and D.A. Laufer, 1987. ^{13}C nuclear magnetic resonance study of acid-base tautomeric equilibria. J. Chem. Soc., Perkin Trans., 2: 453-460.

Sheldrick, G.M., 1997. SHELXTL-PC (version 5.1), Siemens Analytical Instruments Inc., Madison, Wisconsin, USA.

Shingo, M., N. Eiji and T. Yakasaki, 2003. Efficient solid-phase synthesis of 2,1,3-benzothiadiazin-4-one 2-oxides with synphasetm lanterns. Bull. Korean Chem. Soc., 24: 389-390.

Siemens, SMART and SAINT, 1996. Siemens Analytical X-ray instruments Inc., Madison, Wisconsin, USA.

Skopp, G., G. Schweuker, 1984. Separation of cardanols by reversed phase HPLC. Planta Med., 50: 529-530.

Stahl, E., K. Keller and C. Blinn, 1983. Cardanol, a Skin irritant in piuk pepper. J. Med. Plant Res., 48: 5-9.

Tougu, V., 2001. Acetylcholinesterase: Mechanism of catalysis and inhibition. Curr. Med. Chem.-Central Nervous System Agents, 1: 155-170.

Williams, D.A., L. Da. S.L. Sternberg and C.R. Hughes, 2002. Characterization of microsatellite loci in an invasive species, the Brazilian pepper, *Schinus terebinthifolius*. Mol. Ecol. Notes, 2: 231-232.