Pak. J. Bot., 40(6): 2553-2559, 2008.

CHEMICAL CONSTITUENTS OF *TAMARINDUS INDICA* L. MEDICINAL PLANT IN SINDH

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Abstract

Thirty two fatty acids, two other compounds 9ß, 19-Cyclo-4 ß4, 4, 14, x-trimethyl-5ά-cholestan-3ß-ol, 24R-Ethyl cholest-5-en, 3β-ol and 12 essential elements viz., Arsenic, Calcium, Cadmium, Copper, Iron, Sodium, Manganese, Magnesium, Potassium, Phosphorus, Lead, and Zinc were isolated from *Tamarindus indica* medicinal Plant. Accumulation of Copper was the lowest in *T. indica* while Potassium present with highest accumulation. Total protein in *T. indicia* was 7.5 to 6.6 %.

Introduction

Tamarindus indicia L., belongs to the Dicotyledonous family Leguminosae Sub Family Caesalpiniaceae, which is the third largest family of flowering plants with a total of 727 genera and 19, 327 species (Lewis *et al*., 2005). Over all 50% of the population in Pakistan is being treated with traditional medicines by almost 50,000 traditional local herbal practitioners and hakims (Zaidi, 1998). *Tamarindus indica* fruit pulp is used for the preparation of beverages in different regions. *In India*, legumes constitute an important food stuff and are an economic source of protein in the diets of economically weaker sections of population (Kumar *et al*., 1991). Some of the wild nuts and seeds used as food in several parts of the world have considerable promise as protein source (Amubode & Fetuga, 1983). Large segments of human population and animals in developing countries suffer from protein malnutrition (Conway & Toenniessen, 1999). They are playing an important role in human nutrition mainly in developing countries (Mohamed & Rangappa, 1992; Yanez *et al*., 1995). *Tamarindus indica* contain high levels of crude protein (31.08) than the levels reported earlier (Ishola *et al*., 1990) Bhattacharya *et al*., 1994; Siddhuraju *et al.*, 1995). *Tamarindus indica* also contains a high level of protein with many essential amino acids which help to build strong and efficient muscles *T. indica* is also high in carbohydrate, which provides energy, rich in the minerals, potassium, phosphorus, calcium and magnesium *T. indica* can also provide smaller amounts of iron and vitamin A. *T. indica* is an important food resource for the Thai population The flower and leaf are eaten as vegetables (Prakash, 1988). *T. indica* is a plant widely used in traditional medicine in Africa for the treatment of many diseases such as fever, dysentery, jaundice, gonococci and gastrointestinal disorders (Kheraro & Adam, 1974; Kobayashi *et al*., 1996; Ferrara, 2005). Pharmacological investigations on *T. indica* extracts reported them to have antibacterial, antifungal (Pousset, 1989), hypoglycaemic, cholesterolemic (Nabawyia *et al*., 1997),

cytotoxic (Kobayashi *et al*., 1996), anti-inflammatory (Rimbau *et al*., 1999), gastrointestinal (Coutino-Rodriguez *et al*., 2001), hypolipomic and antioxidant activities (Ferrara, 2005; Martinello *et al*., 2006). The phytochemical examination of the methanolic extract of the leaves of *T. indica* afforded two triterpenes i.e., lupanone and lupeol. Both compounds (metabolites) have been isolated for the first time from *T. indica* (Shehla Imam *et al*., 2007). During the present study, fatty acids composition of medicinal plant *T. indicia* from the different areas of Sindh, Pakistan was evaluated to analyze the saturated and unsaturated fatty acids composition. Analysis was carried out for elements viz., As, Ca, Cd, Cu, Fe, K, Mg, Mn Na P, Pb and Zinc and total protein.

Materials and Methods

Collection of plant material: *Tamarindus indica* was collected from Distt Jamshoro, Nawabshah, Hyderabad during November-December 2006. Reference samples were identified through literature of flora of Pakistan. (Nasir & Ali, 1990). The collected plant materials were washed with tap water followed by distilled water and dried in shade at room temperature for 20 days.

Extraction: The dried plant, were chopped into small pieces and was dipped into 5 liter ethanol (EtOH) for about one month at room temperature. The ethanolic extract was filtered and evaporated under reduced pressure at below 40°C using rotary evaporator which yielded dark green thick residue. The extract was then partitioned with Ethyl acetate (EtOAc) and water, and this procedure was repeated 3 times. The EtOAc extract was evaporated under pressure which yielded thick greenish residue.

Column chromatography (CC): The residue containing fatty acids fraction was separated on chromatographer over silica gel (70-230 mesh Merck) column. The column was first eluted with n-hexane and thereafter chloroform was added in order of increasing polarity. First fraction was eluted with pure hexane, fraction "A" was eluted from hexane: chloroform (85:15), fraction "B" from hexane: Chloroform (80:20), fraction "C" from hexane: Chloroform (75:25), and fraction "D" from hexane: Chloroform (70:30).

Esterification. All fractions (A-D) were etherified with diazomethane, 0.5 mg of each fraction was dissolved in MeOH and 0.5 ml of diazomethane was added. The reaction mixture was kept overnight at room temperature (28°C) and was then evaporated .The methylated fatty acid fractions were analyzed first by GC and finally by GC-MS.

Identification

Gas Chromatography-Mass spectrometry (GC-MS): The fatty acids analysis was performed on JEOL JMS 600H Agilest 68 g ON, equipped with 30 m×0.32 mmHP-5 column, stationary phase coating 0.50µm. The column temperature was kept at 250˚C for 2 min., with increase @ 5˚C per min up to injector temperature 250˚C, split ratio 1:35, the carrier gas (Helium) flow rate 1.8ml/min.

Conventional digestion method (CDM)

Elemental assay: The samples were investigated for elemental analysis by using atomic absorption spectrophotometer (AAS), Hitachi Ltd. 180-50.S.N5721. Appropriate working standard solution was prepared for each element. The calibration curves were obtained for concentration vs. absorbance. The data were statistically analyzed by using fitting of straight line by least square method. All elements were determined in medicinal plants under this investigation procedure. A blank reading was also taken, (Kazi, 1993).

Total protein by kjeldha: The sample was digested in (30 ml) H_2SO_4 in the presence of catalyst, CuSO4, (1g) and K_2SO_4 (10g). After digestion Sodium hydroxide (NaOH, 33%) were added followed by steam distillation, the distillate was collected in 20 ml boric acid (4%). Then nitrogen content was determined by using titration with HCl (0.01 N). Indicters used in titration Bromocrecol, green and Methyl red, A factor of 6.25 was used to evaluate total protein concentration following the protocol of ISI-24-1-e (Khanzada *et al.*, 2007).

Results and Discussion

The GC-MS of the methyl lated fatty acids (FAs) revealed the presence of 21 saturated and 11 unsaturated fatty acid (UFAs and two different compounds 9ß, 19-Cyclo-4 ß4, 4, 14, x-trimethyl-5ά-cholestan-3ß-ol and 24R-Ethyl cholest-5-en, 3β-ol were isolated (Table 1). The saturated fatty acid (SFAs) were present in higher amount (67.5 4%) than unsaturated fatty acid, UFAs (30.15%) (Table 2) and other compounds were isolated in very low quantity (2.3%). The largest amount of SFAs is 14.5% n-Heptadecanoate, 13.00% Hexadecanoic acid and n-Nonadecanoate, 6.1% n-Octadecanoic, 5.00% Methyl-n-Pentacosanoic 4.45%, n-Tetradecanoate 4.2 %, n-Heptacosanoate 4.1% and then smaller amount %, n-Nnacosanate 3.09%, Methyl-pentadecanonic 2.6%, Nonanoic acid 1.92%, Nonacosatrienoic acid 1.77%, n-Nonanoate 1.56%, n-Hxocosanoate 1.54%, n-Tridecanoic 1.2%, Methyl-n-tricosanoate 1.1%, n-Docosanoate 1.00 % and these (SFAs) smallest % were isolated, n-Eicosenoate 0.91%, Detricasonic 0.27%, 1-Octanoate 0.3%, Methyl-n-heptanoa 0.2%, The largest amount % of (UFAs) is Nenodecenoic acid 9.2 %,10-Octadecenoicacid 7.8%, Heptadacanoate 3.3%, n-Pentacosenoic acid 2.54%, Heptadecadienoat 1.2%, Methyl-17, 18- hexacosenate, 1.27% -n-dotriacontanoate, 1.72% Pentadecatrienoat, Tetracosadienoate 0.91%, 9-Decenoate 0.8 %, n-Hexacoseoic acid 0.7 %. The GC. mass spectra showed the presence of (SFAs) and (UFAs) methyl ester. Their details are given in Tables 1 and 2 which indicate the presences of 32 different (FAs) and also show the relative retention time (RRT) and relative percentage of occurrence of their methyl ester The detected levels of antinuatritional fatty acid behenic acid in *Tamarindus indica* (5.03%) is in agreement with earlier reports in the same species (Siddhuraju *et al*., 1995). Presence of high levels of unsaturated fatty acids in all the presently studied tribal pulses are nutritionally desirable and also are comparable with some edible legumes like Goa bean and Soybean (Rao & Belavady, 1979). In the present investigation 32 fatty acids were isolated from *Tamarindus indica* and M. Pugalenthi *et al*., (2004) detected only 8 fatty acids. Robert S. Glew *et al*., (2005) have isolated 17 fatty acids from same plant.

Table 1. Fatty acids of *Tamarindus indica* analyzed as Methyl ester.

S. No.	Systematic name	Common name	Molecular	Mol.	R.R.T	Rel.

			formula	Wt.		% age
	Saturated fatty acids methyl ester					
1.	Methyl-n-heptanoa	Methyl heptylate	$C_8H_{16}O_2$	144	16.92	.2
2	1-Octanoate	Caprylate	$C_9H1_8O_2$	158	17.03	.3
3.	n-Nonanoate	Nonylate	$C_{10}H_{20}O_2$	172	19.58	1.56
4.	Nonanoic acid	Laurate	$C_{13}H_{26}O_2$	214	50.53	1.92
5.	n-Tridecanoic	Tridecylate	$C_{14}H_{28}O_2$	228	21.67	1.2
6.	n-Tetradecanoate	Myrislate	$C_{15}H_{30}O_2$	242	24.58	4.2
7.	Methyl-pentadecanonic	Pantadecylate	$C_{16}H_{32}O_2$	256	26.08	2.6
8.	n-Hexadecanoate	Palmilate	$C_{17}H_{34}O_2$	270	29.08	13.00
9.	n-Heptadecanoate	Margorate	$C_{18}H_{36}O_2$	284	30.52	14.5
10.	n-Octadecanoic	Stearate	$C_{19}H_{38}O_2$	298	32.65	5.00
11.	n-Nonadecanoate	Nonadecylate	$C_{20}H_{40}O_2$	312	33.88	6.1
12.	n-Eicosenoate	Arachidate	$C_{21}H_{42}O_2$	326	35.97	.91
13.	n-Docosanoate	Behenate	$C_{23}H_{46}O_2$	354	39.13	1.00
14.	Methyl-n-tricosanoate		$C_{24}H_{48}O_2$	368	40.17	1.1
15.	n-Tetracooosano	Lignocerate	$C_{25}H_{50}O_2$	382	41.62	1.54
16.	Methyl-n-Pentacosanoic	Methyl-Pentacosanoate	$C_{26}H_{52}O_2$	396	43.25	4.45
17.	n-Hxocosanoate	Cerotate	$C_{27}H_{54}O_2$	410	45.1	1.45
18.	n-Heptacosanoate		$C_{28}H_{56}O_2$	424	37.8	4.1
19.	Nonacosatrienoic acid		$C_{29}H_{52}O_2$	432	51.92	1.77
20.	n-Nnacosanate	Lignocerate	$C_{30}H_{60}O_2$	452	53.53	3.09
21.	Detricasonic		$C_{32}H_{64}O_2$	480	65.73	0.27
	Total					**67.54**

Table 2. Unsaturated fatty acid of *Tamarindus indica*.

	Unsaturated fatty acid methyl ester					
1.	9-Decenoate		$C_{11}H_{20}O_2$	184	17.82	.8
2.	Pentadecatrienoate		$C_{16}H_{26}O_2$	250	27.5	.91
3.	Heptadacanoate		$C_{18}H_{30}O_2$	278	34.23	3.3
4.	Heptadecadienoat		$C_{18}H_{32}O_2$	280	37.48	1.2
5.	10-Octadecenoicacid	Oleate	$C_{19}H_{36}O_2$	296	32.22	7.8
6.	Nenodecenoic acid		$C_{20}H_{38}O_2$	310	33.45	9.2
7.	Tetracosadienoate		$C_{25}H_{46}O_2$	378	56.58	.91
8.	n-Pentacosenoic acid		$C_{25}H_{48}O_2$	380	42.18	2.54
9.	n-Hexacoseoic acid		$C_{26}H_{50}O_2$	394	43.78	.7
10.	Methyl-17, 18- hexacosenate	Methyl hexacosenoate	$C_{27}H_{52}O_2$	408	45.83	1.27
11.	Methyl-n-dotriacontanoate		$C_{33}H_{64}O_2$	492	48.67	1.72
	Total					**30.15**
	Different compounds					
1.	24R-Ethyl cholest-5-en, 3β-ol	B-sitosterol	$C_{29}H_{50}O_2$	414	57.61	1.2
2.	9ß, 19-Cyclo-4 ß4, 4, 14, x-trimethyl-5ά-cholestan-3ß-ol	Cycloartanol	$C_{30}H_{52}O$	428	55.8	1.1
	Total					**2.3**

21 Saturated, 11 Unsaturated, 2 Different compounds. Total compounds=34 Total %age of Saturated + Unsaturated fatty acid different compound = 99.99

(Mol.wt= Molecular weight, R.R.T= Relative retention time, Rel % age = Relative percentage

Total protein analysis: The total protein contents isolated of leaves and flowers of *Tamarindus indica*: Jamshoro 15.6%, Nawabshah Distt, 10.8%. Hyderabad Distt, 8.7%.

The total protein content is found to be lower when compared to an earlier report in the same species of *T. indica* 14% (Arinathan *et al*., 2003). Seed protein 6.9% (***M. Pugalenthi et al.,*** 2004).

Elements analysis: Copper 0.76, Iron 14.07. Cadmium 3.36, Arsenic 54.25 µg, Zinc 8.52, Lead 0.27, Sodium 10.9, Potassium7.16, Calcium 20.2, Magnesium 60.1, Manganese 25.9, Phosphorus 20.4, ppm. The maximum element ratio Magnesium 60.1 is present in*T.indica* and minimum ratio in Copper. 0.76. If we compare with others, *T.Indicia* registers the lowest level of Sodium content but it is seems to be higher compared to an earlier report in the same species (Ishola *et al.,* 1990); Sodium 28.83 Calcium 315.28 Potassium 248.56, Magnesium 285.14 Phosphorus 369.47, Iron 7.14, Copper 0.59, Zinc 6.94 Manganese 0.81 (*M. Pugalenthi et al.***,** 2004). Calcium, 101, Magnesium 71.0, Iron 2.0 Copper 2.0, Sodium 8.0 Potassium (mg) 270.0 (Lewis *et al*., 1964; Anon., 1976; Duke 1981) detected mineral composition. Calcium, 36.6, Copper 2.10, Manganese 12.1, Sodium 8.90, Iron 45.5, Zinc 7.00, Potassium 1308, Magnesium, 104, (I.A. Ajayi *et al*., 2006). *T. indica* seeds have Calcium, 10.00. Potassium 21.0. Sodium, 2.1. Magnesium, 15.0. Iron, 75.9. Phosphorus, 25.5 (Yusuf *et al*., 2007) and Copper 9.09, Manganese 215, Magnesium, 1.153, Sodium, 62.1, Iron 31.7, Zinc 13.2, lead 0.1, Potassium 6.54 (Robert S.Glew *et al.,* 2005).

Table 3. Concentrations of elements detected in *Tamarindus indica* L., in Sindh, Pakistan.

S. No	Elements	Symbol	Mg/kg
1.	Manganese	Mn	25.9
2.	Calcium	Ca	20.2
3.	Phosphorus	P	30.4
4.	Sodium	Na	10.9
5.	Arsenic	As	54.25µg/kg
6.	Iron	Fe	14. 07
7.	Zinc	Zn	8.52
8.	Potassium	K	7.16
9.	Lead	Pb	0.27
10.	Cadmium	Cd	3.36
11.	Copper	Cu	0.76
12.	Magnesium	Mg	60.1

Conclusion

Our study showed that there is great variation in fatty acids, elemental composition and total protein in *T. indeca* (Tables 1, 2, 3).

More over As, Pb, Cd were not detected by previews works and in recent investigation 32 fatty acids were isolated from same plant. The total protein analysis of *T. indica* medicinal plants from Jamshoro Distt. (Sindh)15.6%. The great variation of fatty acids, elements and total protein is due to the environmental and ecological factors.

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(Received for publication 22 January 2008)