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PHYTOCHEMICAL STUDIES ON *PITHECELLOBIUM DULCE BENTH.* A MEDICINAL PLANT OF SINDH, PAKISTAN

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Abstract

In the present study, the seed extracts of *Pithecellobium dulce Benth.* was subjected for fatty acid analysis and 9 saturated and 17 unsaturated fatty acids were identified by (GC MS). Moreover, some essential and toxic elements (e.g. As, Cu, Cd, Fe, K, Mg, Na, Pb & Zn) were also measured in different concentrations (Zn & K being the highest (26.89 mg/kg) and Pb (0.19mg/kg) & As (17.6µg/kg) were the lowest in concentrations) justifying its medicinal applications. Total protein contents analyzed in different parts of *P. dulce* was highest in seeds ranging from 50.3-67.1%, stems 15.7%, roots 10.6%, leaves 13.7%, flowers 14.8%, and in fruits 10.50% as established by kjeldhal method. *P.dulce* plant was found to be a rich source of proteins, fatty acids and essential elements particularly seeds and can be exploited for human and animal consumptions.

Introduction

Medicinal plants play the most important role in the traditional medicines in various developing countries. Most of the flora remain virtually of the medicinal utilizing through traditional eastern system of medicines strongly upholds the use of elements for curing many diseases (Kaneez *et al.,* 1998). The steroid saponin, lipids, phospholipids, glycosides, glycolipids and polysaccharides have been reported as a seed extract from various plants (Shyam & Chittranjan, 1971; Misra *et al*., 1979). The studies on alkylated resins from seed oil have been reported recently (Sugumaran, 2008;Banarjee, 2005). It is evident that the plant has great potentials in treating a number of ailments where the free radicals have been reported to be the major factors contributing to the disorders (Aruoma, 1998). Proteinaceous inhibitors have also been purified and characterized from a variety of seed plant sources (Hilder *et al*., 1989; Richardson, 1991; Vemekar *et al*., 1999; Bode & Huber, 2000; Franco *et al*., 2002; Macedo *et al*., 2004; Araujo *et al.*, 2005; Rackis *et al*., 1986).

Pithecellobium dulce Benth., belonging to the family of *Leguminosae* (*subfamily Mimosoideae*) locally known as Jangal Jalebe and with English name as Manila Tamarind, is a small to medium sized, evergreen, spiny woody legume tree up to 18 m height, it is native of tropical America and also found throughout India and Pakistan. *P. dulce Benth* grows abundantly in the Sindh province of Pakistan but its scientific utility is hardly tested. Reportedly active against venereal diseases. The bark of the plant which contains 37% of catechol type tannins is reported to be used as febrifuge, dermatitis and eye inflammation (Pithayanukul *et al*., 2005). Seeds are particularly rich in proteins and peptides and having potential to combat protein malnutrition. The decoction is also given as excellent treatment for anemia. The constituents of *P. dulce* fruits have been isolated and characterized (Nigam *et al*., 1962). The anti-inflammatory activity due to saponin fraction of *P. dulce* fruits (Bhargvakrishna *et al*., 1970) was also studied. On the other hand, quericitin, kaempferol, dulcitol and afezilin have been reported from the leaves (Adinarayana *et al*., 1985; Zapesochnaya *et al*., 1980; Sugumaran *et al*., 2008a-b). Many other parts of *P.dulce* have also been used traditionally to treat diseases, such as skin of the stem for dysentery, leaves for intestinal disorders, and seeds for ulcers, (De Lumen *et al*., 1986; Sotelo *et al*., 1990). The powders and methanolic and/or aqueous extracts of *P. dulce* seeds have proved fungistatic and possess fungicidal effects against plant pathogens (Zapesochnaya *et al*., 1980). Seeds of *P. dulce* have high contents of proteins, dietary fiber, and unsaturated fatty acids (Norioka *et al*., 1988; Franco & Melo, 2000). Legume seeds also contain anti-nutritional compounds such as the protease inhibitors (Pernas *et al*., 2000). Inhibitory proteins involved many protease inhibitors and have shown biological effects ranging from anti-nutritional to beneficial (Richardson, 1991; Bode & Huber, 2000; Vemekar *et al*., 1999; Franco *et al*., 2002; Macedo *et al*., 2004; Al-Wasaly *et al*., 1995).

In the present study, the seed extracts of *Pithecellobium dulce* were subjected for the determination of fatty acids composition using by GC MS. 9 saturated and 17 unsaturated fatty acids have been identified and quantified. The essential elements such as As, Cu, Cd, Fe, K, Mg, Na, Pb, and Zn have also been analyzed in variable ranges which also showing the medicinal important of plant. The total protein content in different parts of *P. dulce* was also measured by using (kjeldhal method) reveal very high concentrations suggesting its possible utilization for human and/or animal consumptions.

Materials and Methods

Collection of plant materials: The plant materials of *Pithecellobium dulce* Benth. were collected from the area of University of Sindh, Jamshoro during June-July 2008, reference sample were identified through Flora of Pakistan (Ali, 1973). The collected plant material was washed with tap water followed by distilled water and dried in shade at room temperature for 15-20 days (Khanzada *et al*., 2008a,b).

Fatty acid extractions & identification: Dried plant was chopped into small pieces and was dipped into two 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 gummy residue. The extract was then partitioned with ethyl acetate (EtOAc) and water.The ethyl acetate subjected to fraction chromatography 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 (90:10), fraction "B" from hexane: chloroform (85:15), fraction "C" from hexane: chloroform (75:25), and fraction "D" from hexane: chlorofrom (70:30). All the fractions were esterified with diazomethane, 0.5 mg of each fraction was dissolved in Methanol (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 ethylated fatty acids were finally analyzed and identified by GC-MS. The analysis was performed on JEOL JMS 600H Agilent 6890N, equipped with 30 m×0.32 ZP-5MS column, stationary phase coating 0.25µm. The column temperature was kept at 70˚C for 2 min with an increase at the rate of 4˚C per min up to 260˚C. Injection temperature 250˚C, split ratio 1:45, the carrier gas (Helium) flow rate 1.0 ml/min (Khanzada *et al*., 2008b).

Elemental assay: The samples were investigated for elemental analysis by using atomic absorption spectrophotometer (Hitachi Ltd, Japan). Appropriate working standard solution (180-50.S.N5721) 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. A blank reading was also taken (Khanzada *et al*., 2008a-b).

Total protein analysis by kjeldhal method: The sample was digested in H_2SO_4 (30 ml) in the presence of catalyst $CuSO_4$ (1g) and K_2SO_4 (10g), after digestion Sodium hydroxide (NaOH, 33%) was 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 (0.01N). A factor of 6.25 was used to evaluate total protein contents (Khanzada *et al*., 2008b).

Results and Discussion

The nutrients essential for life are proteins, fats and carbohydrates, all contribute to caloric content of the dietary, minerals including trace elements, vitamins and water (Underwood, 1994). The quality and quantity of protein in the seed are basic factors in the selection of plants for nutritive value, systematic classification and plant improvement programs (Siddique, 1998). Most population of the world is facing malnutrition problems. In Pakistan the protein gap would continue to increase unless well-planned measures are adopted to tackle the situation. It is therefore imperative to increase protein production by utilizing all the available ways and means. In addition, increase in conventional production much work has been done in recent years in order to develop new chemical and biological methods for the production of protein foods and feeds (Shah & Khalil, 1988). Exploration and utilization of unconventional legumes are promising to fulfill the deficiency of proteins and essential fats in human nutrition as evidenced legumes (Apata & Olighobo, 1994; Badifu, 1994; Madubuike *et al*., 1994; Ezeagu *et al*., 1996; Petzke *et al*., 1997). Protein contents also contribute to the formation of hormones which controls a variety of body functions such as growth, repair and maintenance of other body proteins (Mau *et al.,* 1999). Leaves can be used as a plaster to allay pain even from venereal sores, and can relieve convulsions (Sugumaran, 2008a-b). (Nandkarni, 1982). Furthermore, chemical investigation on the different parts of the plant has resulted in the isolation of a large number of novel and interesting metabolites. Some of the compounds have been screened for bioactivity (Chandran & Balaji, 2008). In view of the importance of saponins, as possible spermicidal agents, the saponins of *P. dulce* were also subjected to tests for spermicidal property by Banarjee (2005). The sapogenin showed the activity in the dilution of 0.03% against human semen (Misra *et al.,* 1979; Delgado *et al.,* 2004). Delgado *et al*., (2004). Pithayanukul *et al*., (2005). This isoflavonoid was isolated from root extract (Banarjee, 2005). Studied the fatty acids distribution by GLC in total lipids, phospholipids and glycolipids of this plant. The major fatty acids present are palmitic, stearic, oleic, linoleic, myristic, linolenic and arachidic acids. (Rzedowski *et al*., 1985). Oils and fats are an important source of energy for the human diet and also contribute significantly to the sensory characteristics of food (Cahoon *et al.,* 2009). Watermelon seed protein the high-quality dietary and purposeful property of proteins, such as used in food formulations property recently reported by (Ali Abas Wani 2011). In the present study, the extract of *P. dulce* Benth., were analyzed for fatty acid composition and 9 saturated and 17 unsaturated fatty acids (total 26) have been isolated and characterized. The major fatty acids isolated from this source is Palmilate 7.75% and Oleate 6.89% as saturated fatty acids, *P. dulce* seed oil fatty acids as reported previously by (Khatri, 1995). The recently reported (Katekhay, 2013) extract from *P. dulce* bark prohibited strong α-glucosidase, α-amylase inhibitors and controlling for suppressing post-prandial hyperglycemia and helpful treatment for diabetes mellitus. The major fatty acids were oleic acid and linoleic, Erucic acid. While in unsaturated fatty acids heptadectrienote (9.36%) is in higher concentrations (Tables 1 and 2). Total percentage of saturated fatty acid is observed to be 36.93% and unsaturated fatty acids as total percentage of 63.06% which is higher than saturated fatty acid. Table 3 summarizes the concentrations (mg/kg) of essential and toxic elements, which were observed in different concentrations As, Cu, Cd, Fe, K, Mg, Na, Pb, and Zn have been analyzed for *P. dulce*. In highest range Zn & K, 26.89 mg/kg and Pb, 0.19mg/kg, As, 17.6 µg/kg was the lowest range in *P.dulce.* Total protein contents in seeds of *P. dulce* ranged between 50.30-67.11, while stems, roots and leaves contains, 15.72%, 10.58% and 13.75% respectively and flowers and fruits 14.76% and 10.50% respectively. The seeds of plant (*P. dulce)* are rich sources of protein.

Table 1. Saturated Fatty acids methyl ester. *Pithecellobium dulce* Benth.

S.No.	Systematic name	Common name	Moleculr formula	Mol. Wt.	R.R.T	Rel. % Age
1.	n-Tridecanoate	Tridecylat	$C_{14}H_{28}O_2$	228	25.88	5.34
2.	n-Tetradecanoate	Myrislate	$C_{15}H_{30}O_2$	242	29.33	1.78
3.	Hexadecanoate	Pantadecylate	$C_{16}H_{32}O_2$	256	29.88	6.03
4.	n-Hexadecanoate	Palmitate	$C_{17}H_{34}O_2$	270	39.52	7.75
5.	n-Heptadecanoate	Margorate	$C_{18}H_{36}O_2$	284	33.45	2.93
6.	n-Octadecanoate	Octadecanoate	$C_{19}H_{38}O_2$	290	36.83	3.85
7.	n-Hxocosanoate	Cerotate	$C_{27}H_{54}O_2$	410	53.3	1.90
8.	Nonacosatrienoate	Nonacosatrienoate	$C_{30}H_{60}O_2$	452	43.28	2.07
9.	Tetratriacontanoate	Geddlicacid	$C_{34}H_{68}O_2$	536	44.4	5.28
	Total					**36.93**

, Saturated, 17, Unsaturated Total compounds = 26.

Total % age of Saturated + Unsaturated fatty acid = 99.99

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

Table 2. Unsaturated fatty acid methyl ester *in Pithecellobium dulce* Benth.

S.No.	Systematic name	Common name	Molecuar formula	Mol. Wt.	R.R.T	Rel. % Age
1.	n-Heptaecenoate	n-Heptaecenoate	$C_{17}H_{32}O_2$	208	19.93	0.41
2.	Tridecatrienoate	Tridecatrienoate	$C_{14}H_{22}O_2$	222	19.78	1.21
3	Methyl-2-Tridecynote	Tridecynote	$C_{14}H_{24}O_2$	224	31.77	3.45
4.	Methyl tricosenoate	Decylacrylate	$C_{14}H_{26}O_2$	226	25.7	5.23
5.	2,4,5-Tetradecatrienoate	Tetradecatrienoate	$C_{15}H_{24}O_2$	236	25.42	2.01
6.	7-Ethyl-3-Methyl-2, 6-undecadienoate	Undecadienoate	$C_{15}H_{26}O_2$	238	18.85	4.14
7.	Pentadecatrienoate	Pentadecatrienoate	$C_{16}H_{26}O_2$	250	23.93	2.18
8.	Hexadecadienoate	Hexadecadienoate	$C_{16}H_{28}O_2$	252	26.03	2.30
9.	n-hexadecanoate	Plmitoleate	$C_{17}H_{32}O_2$	268	27.15	2.47
10.	Heptadectrienoate	Heptadectrienote	$C_{18}H_{32}O_2$	278	28.68	9.36
11.	Heptadecadienoate	Heptadecadienoate	$C_{18}H_{34}O_2$	280	30.07	4.02
12.	Heptadecenoate	Heptadecenoate	$C_{18}H_{38}O_2$	282	33.07	4.54
13.	9, 12, 15, Octadecatrienoate	Octadecatrienoate	$C_{19}H_{34}O_2$	292	29.07	2.76
14.	10-Octadecenoate	Oleate	$C_{19}H_{36}O_2$	296	56.22	3.03
15.	n-Octadecanoate	Stearate	$C_{19}H_{38}O_2$	298	32.33	6.89
16.	Eicosatrienoate	Eicosatrienoate	$C_{20}H_{34}O_2$	306	33.2	4.19
17.	Methyl-17, 18- hexacosenate	hexacosenoate	$C_{27}H_{52}O_2$	408	42.82	4.88
	Total					**63.06**

9, Saturated, 17, Unsaturated Total compounds =26.

Total % age of Saturated + Unsaturated fatty acid = 99.99

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

Table 3. Elemental composition (Essential and/or toxic elements) of *Pithecellobium dulce* Benth.

S.No.	Elements	Symbol	Concentrations (mg/kg)
1.	Arsenic	As	17.6 µg/kg
2.	Copper	Cu	16.25
3.	Cadmium	Cd	3.48
4.	Iron	Fe	1.89
5.	Lead	Pb	0.19
6.	Magnesium	Mg	15.06
7.	Potassium	K	26.89
8.	Sodium	Na	10.19
9.	Zinc	Zn	26.89

Conclusion

Chemical investigation on the different parts of a medicinally important plant and an alternative food source for humans and animals, (i.e. *Pithecellobium dulce Benth*.) have been performed resulted in a great variation in fatty acids, elemental composition and total protein contents as compared to other legumes (Tables 1-3). In conclusion, *P. dulce* plant was found to be a rich source of proteins, fatty acids and essential elements particularly seeds and can be easy exploited as alternative food source for human and animal consumption.

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