

Spectrometric Detection of Organic Compounds and Toxicity of Ethanolic Leaves Extracts of *Prosopis Juliflora*

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Abstract

Spectrometric detection of saponins, tannins and alkaloids in *Prosopis juliflora* using Fourier Transform Infrared (FTIR) spectroscopy is reported in this paper. Crude dry plant powders and corresponding ethanolic extracts were mixed with potassium bromide (KBr) powder and compressed to a thin pellet for infrared examination. The plant powders, ethanolic extracts and leaves ethanolic extract (LEE) fractions exhibited characteristic infrared spectra due to various functional groups –OH, –C=O, C-H, and C=C absorptions. Phytochemical analysis confirmed the presence of saponins, tannins and alkaloids in the tested specimens. *Prosopis juliflora* whole plant parts were used as a reference sample. Toxicity and safety levels of *P. juliflora* were also investigated. Acute and sub-acute toxicity of *P. juliflora* ethanolic leaves extract was evaluated using *Swiss albino* rats. All clinical signs and symptoms were recorded within 24 hours. These results indicated that direct detection of bioactive compounds was possible by infrared analysis. Toxicity symptoms were moderately observed and post mortem did not show any major gross effects on the internal organs.

Keywords: *Prosopis juliflora*, infrared examination, potassium bromide, acute and sub-acute toxicity, clinical signs, doses, symptoms.

INTRODUCTION

Plants are used for various purposes in different parts of the world. Their usefulness can be in the form of food, shelter, textile, medicine and many more (Fabeku, 2006). Exploring the pharmacological potentials of plants for both preventive and curative therapies is an age old concept. Records of indigenous knowledge from various parts of the world illustrate an age long tradition of plant being a major bio-resources base for health care. *Prosopis juliflora* is an evergreen tree native to northern South America, Central America and the Caribbean (Pasicznik *et al.*, 2004). It is fast growing, nitrogen-fixing and tolerant to arid conditions and saline soils (Anonymous 2003, Pasicznik *et al.*, 2004). In Kenya *P. juliflora* was first planted in the beginning of the 1970s to rehabilitate a quarry in Bamburi near Mombasa (Ebenshade & Grainger 1980, Maghembe *et al.*, 1983). In the early 1980s *P. juliflora* was introduced in the Lake Baringo area through the Fuelwood Afforestation Extension Project (FAEP), the major objectives being involving the local people in tree planting to overcome problems such as lack of firewood and overgrazing (Kariuki 1993, Lenachuru 2003).

Prosopis juliflora pods which are high in protein and sugars may be important fodder for livestock, and / or food for humans. In the semi-arid areas of north eastern Brazil (de Barros *et al.*, 1988), it partly offsets fodder scarcity during the dry season. Results of feeding trials indicate that rations for goats, sheep, beef cattle and dairy cattle can give very good weight gains and/or milk production when about 60% of the diet consists of ground *Prosopis* pods. The flowers, leaves and fruits are reported to contain protein, fat, carbohydrate, fiber, Calcium, and Phosphorus (FAO, 1992). Analysis of the fruit also reveals presence of tannin-like material. Mesquite gum readily hydrolyses with dilute sulfuric acid to yield L-arabinose, D-galactose and 4-o-methyl-D-glucuronic acid at 4:2:1. Owing to the high content of arabinose, the gum is an excellent source of sugar. Roots contain 6–7% tannin, bark 3–8.4%, and dry wood 0.9%.

Prosopis juliflora is known for its medicinal value and many alkaloids such as juliflorine, julifloricine and julifloridine (Ahmad *et al.*, 1978), juliprosine (Daetwyler *et al.*, 1981), juliprosinine and juliflorinine (Ahmad *et al.*, 1989 a, 1989b), benzene insoluble alkaloidal fraction (containing two major and three minor alkaloids) (Ahmad *et al.*, 1989a, 1989b) have been isolated and their biological activities *in vitro* have been demonstrated. Antifungal, plant growth inhibiting and DNA binding alkaloids such as tryptamine, piperidine, phenethylamine and juliprosopine and their isomers have been isolated from the leaves of *P. juliflora* (Tapia *et al.*, 2000). The alkaloids 5-hydroxytryptamine and tryptamine are reported from this species (Simpson, 1977). *P. juliflora* leaves are commonly used to treat eye conditions, open wounds and dermatological ailments. Acting much as antacid, it can also treat digestive problems. It has soothing, astringent, and antiseptic properties (Davidow, 1999). The leaves are also reported to contain crude protein levels of 14-22%, crude fibre 21-23%, nitrogen free extract 43-50%, calcium 1.5% and phosphorus 0.2% while mineral content is directly related to the levels of minerals in the soil (Pasicznik *et al.*, 2001). Bark extractives exhibit antifungal properties (Càceres *et*

al., 1995). The remarkable economic and physiological characteristics of *P. juliflora* make it a prime contributor to the development of many arid regions, especially if its invasive habit is controlled and the thorns that limit its widespread acceptance are controlled. Efforts are underway in different parts of the world to moderate these unwanted attributes. New erect *Prosopis* clones with small thorns and high production of highly palatable pods have been identified in Peruvian field trials (Felker, 2003). Toasted seeds are added to coffee. Bark, rich in tannin, is used for roofing in Colombia. The gum forms adhesive mucilage, used as an emulsifying agent in confectionary and mending pottery. Roots contain 6–7% tannin, which might discourage Rhizobia (Bressani, 1977). *Prosopis* leaves contain various chemicals known to affect palatability to livestock, but also suppress the germination and growth of crops, weeds and other trees.

Owing to growing demand for herbals, the current need is to intensify research in the field of medicinal herbs and to get authentic information on the subject. This study aimed at investigating the phytochemical properties of the leaves, pods, barks and roots extracts from sequentially extracted samples using solvents of differing polarities and evaluate the acute and sub-acute toxicity in rats.

2.0 Materials and methods

2.1 Collection and identification of plant specimens

The plant specimens were collected from Marigat district of Rift Valley Province, Kenya. The voucher specimens were deposited in the Botany department laboratory of Jomo Kenyatta University of Agriculture and Technology. Authentic samples were air-dried under the shade and ground into a fine powder, and stored in airtight plastic containers.

2.2 Phytochemical screening

This was performed using standard procedures (Sofawara, 1993; Trease *et al.*, 1989; Harborne, 1973; Siddiqui and Ali, 1997). Different extracts were tested for tannins, alkaloids, saponins, flavonoids, steroids, terpenoids, anthraquinones, reducing sugars and glycosides. The results obtained were recorded in tabular form Table 1.

2.3 Infrared Spectra

A little powder of plant specimen was mixed with KBr salt, using a mortar and pestle, and compressed into a thin pellet. Infrared spectra were recorded as KBr pellets on a Shimadzu FTIR Spectrometer 8000 series, between 4000 – 500 cm^{-1} . Infrared absorptions were recorded in tables 2 and 3.

2.4 Acute Toxicity Tests of *P. juliflora* Leaves Ethanolic extracts (LEE)

22 Wistar Albino rats were obtained from random breeding in a closed colony from the animal house at Jomo Kenyatta University of Agriculture and Technology (JKUAT). They were kept in a temperature-controlled environment ($23 \pm 2^\circ\text{C}$) with a 12 h light-dark cycle. Food and water were provided *ad libitum*. They were acclimatized for 7 days before the dose administration. The doses selected were 175mg/kg, 550mg/kg, 1750mg/kg, 2000mg/kg and 5000mg/kg. All the tests were conducted according to OECD 425 Guidelines procedures i.e. “up and down procedures”. The test substances were administered orally in a single dose using an intubation cannula.

3.0 Results

3.1 Phytochemical Test

The total extractability of the *P. juliflora* leaves was 12.06% while that of the ethanolic leaves were 6.94%. The extracts were greenish brown, while its consistency was viscous to semisolid on standing. This low yield of the leaves extracts indicates that it is poorly absorbed in the gastrointestinal tract. Table 1 below shows the phytochemical test results for saponins, tannins and alkaloids in the samples under investigation. In quantification, alkaloid were found to contain $4.390 \pm 0.959\%$, saponins was $4.3035 \pm 0.2210\%$ while tannins was $1.051978 \pm 0.0703\%$. The presence of these secondary metabolites is evidenced by the pharmacological use of the leaves extracts. The leaves have been used to make eyewashes to treat pink eye. It is also used to treat Intestinal problems such as diarrhea and empacho (Davidow, 1999). They can also be prepared to treat headaches, painful gums and bladder infection. A poultice of leaves is used for red ant stings. Leaves can serve as an emetic or system cleanser (Kay, 1996).

3.2 Infrared Spectra

The Infrared absorptions results were recorded in tables 2 and 3.

3.3 Acute toxicity study

During acute toxicity test, *P. juliflora* LEE was administered at dosage levels of 175 mg/kg, 550mg/kg, 1750mg/kg, 2000mg/kg and 5000mg/kg, once orally to five different groups. The test used 22 rats and only two

deaths were observed at 1750mg/kg dosage level. At this dosage level two extra rats per sex were treated to confirm the lethality of LEE and they all survived with only minor observable clinical symptoms of toxicity. No more death was noted even at the higher doses. The no-observed-adverse-effect level (NOAEL) for *P. juliflora* LEE administered was 1750 mg/kg while the minimum lethal dose and the estimated LD₅₀ was > 5000 mg/kg. The clinical signs observed included: - hypo-activity, pilo-erection, loss of appetite, salivation and hyperventilation (i.e. stress symptoms), nasal allergy and general body weakness. These signs also varied depending on the dosage used (i.e. they were more pronounced at the doses of 2000mg/kg and 5000mg/kg) as compared with the other lower doses. All the signs reversed within the first 72 hours observation period. At the end of the observation period all the rats were humanely sacrificed and a gross morphological observation was made on some selected tissues. There were no major variations on their structure and texture. The rat that died was noted to have a bloated stomach, anemic and with mild liver inflammation which could be due to malnutrition. The gastrointestinal tract had signs of mild inflammation. The results are as shown in table 4 below.

Discussions

A special feature of higher plants is their capacity to produce a large number of organic chemicals of high structural diversity. Phytochemical analyses of *Prosopis juliflora* gave positive results for saponins, tannins and alkaloids. These results were confirmed by infrared absorptions recorded. The FTIR spectra for saponins showed characteristic infrared absorbance (Table 2) of the hydroxyl group (-OH) ranging from 3472 cm⁻¹ (F^C) to 3159 cm⁻¹ (SEE); C-H ranging from 2932 cm⁻¹ (*pods powder*) to 2318 cm⁻¹ (F^B); C = C absorbance ranging from 1643 cm⁻¹ (FC) to 1616 cm⁻¹ (REE); C=O ranging from 1724 cm⁻¹ (F^A) to 1701 cm⁻¹ (F^B). Oligosaccharide linkage absorptions C-O-C were evident between 1092 cm⁻¹ (F^A) to 1042 cm⁻¹ (root powder). C=C-C_{str} aromatic absorptions were characterised from 1555 cm⁻¹ (leaves powder) to 1404 cm⁻¹ (LEE).

The existence of one or more aromatic rings in a structure is normally readily determined from the C-H and C=C-C_{str} ring-related vibrations (Coates, 2000) which are common in the organic metabolites investigated. These bioactive components have potential roles in health care and can be used to synthesize chemicals for new drug development. Drugs are derived from substances, such as alkaloids (e.g., caffeine, from the coffee shrub—*Coffea arabica*—used as a stimulan), glycosides (e.g., digoxin and other digitalis glycosides, from foxglove—*Digitalis spp.*—used to treat heart failure), alcohols, esters, aldehydes, or other constituents or mixtures of constituents isolated from the plant or animal (Monika *et al.*, 2008). *Prosopis juliflora* has many alkaloids such as juliflorine, julifloricine and julifloridine (Ahmad *et al.*, 1978), juliprosine (Daetwyler *et al.*, 1981), juliprosinine and juliflorinine (Ahmad *et al.*, 1989 a, 1989b) which have similar infrared functional group absorptions characteristics as above. The above infrared functional group absorptions characteristic of saponins were cited in literature (Kareru *et al.*, 2007, Kirmizigul *et al.*, 2002 among others. Saponins are a large family of structurally-related compounds of steroid or triterpenoid aglycone (sapogenin) linked to one or more oligosaccharide moieties by glycosidic linkage. The presence of both polar (sugar) and nonpolar (steroid or triterpene) groups provide saponins with strong surface-active properties (Makkar *et al.*, 2007). Their physiochemical and biological properties feature structural diversity, which have led to a number of traditional and industrial applications (Martin *et al.*, 1999).

According to Globally Harmonized Classification System for Chemical Substances and Mixtures (GHS), when a main study with starting dose of 5000 mg/kg is used, and there is no mortality observed (no toxicity) then the substance will be considered **unclassified** (OECD 420, 2001) According (Loomis, 1996) and (Pascoe, 1983), the systems for the classification of the toxicity of compounds can be based on their LD₅₀ values. Their classifications are as shown below in table 5.

Table 5: Systems for the classification of the toxicity of compounds

Category	LD ₅₀ (mg/kg)	LD ₅₀ (mg/kg)	Classification
Extremely toxic	1 or less	< 5	Super toxic
Highly toxic	1 to 50	5- 50	Extremely toxic
Moderately toxic	50 to 500	50- 500	Very toxic
Slightly toxic	500 to 5000	500- 5000	Moderately toxic
Practically non-toxic	5000 to 15000	5000- 15000	Slightly toxic
Relatively harmless	More than 15000	> 15000	Practically non-toxic
(Loomis <i>et al.</i>, 1996)		(Pascoe <i>et al.</i>, 1983)	

Similarly, according to OECD TG 423, OECD 425 any substance whose LD₅₀ value is above 5000mg/kg is considered to be **practically non-toxic**. Based on our finding and in comparison to the above classifications, crude powders and leaves ethanolic extracts of *P. juliflora* can be considered to be:

- (i) Slightly toxic according to Pascoe *et al.*, 1983.
- (ii) Practically non-toxic according to Loomis *et al.*, 1996 and OECD TG 423/OECD 425.
- (iii) Unclassified according to GHS.

In general, its LD₅₀ values greater than 5000mg/kg which classify the plant as slightly toxic. This is in agreement

with the traditional usage by the Americans Indians who uses the pods to supplement their beverages and in treatment of skin ailments.

Conclusions

Spectrometric detection of saponins, tannins, alkaloids in *P.juliflora* powders and from their extracts was performed directly using FTIR spectroscopy. The method is versatile, fast, and economical. *Prosopis juliflora* has an LD₅₀ values greater than 5000mg/kg and can be used by both human and animals, with a degree of safety and tolerance.

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Result Tables

Table 1: Phytochemical test results in selected plant parts.

Plant part	Saponins	Tannins	Alkaloids
Leaves			
Ethanollic extract	+	+	++
Aqueous extract	+	+	++
Pods			
Ethanollic extract	+	++	++
Aqueous extract	+	++	++
Roots barks			
Ethanollic extract	++	+	++
Aqueous extract	++	+	++
Stem barks			
Ethanollic extract	++	++	++
Aqueous extract	++	++	++

Table 2: FTIR spectra of plant powders (KBr disc), cm⁻¹

Functional group	Plant samples and wave numbers (cm ⁻¹)			
	Root powder	Stem powder	Leaves powder	Pods powder
O-H _{str}	3418	3422	3337	3352
N-H _{str}	*	2149	*	*
C-H _{str}	2928, 2368	2928, 2388	2924, 2866, 2365	2932
C=C _{str}	1624	1628	1643	1643
C=C-C _{str} Aromatic	1524	1524	1555	1551
C-O _{str} aromatic	1369	1331	1323	*
C-O-C)	1042	1045	1049	1057

*Not observed

Table 3: FTIR spectra of ethanolic extracts and leaves ethanolic extract (LEE) fractions (KBr disc), cm⁻¹

Functional group	Plant samples and wave numbers (cm ⁻¹)							
	REE	SEE	LEE	PEE	F ^A	F ^B	F ^C	F ^D
O-H _{str}	3248	3236, 3159	3398	3395	3387	3387	3472, 3345, 3260, 3190	3209
N-H _{str}	2095	*	*	*	*	*	3140, 3044	2168
C-H _{str}	2923, 2228,	2928, 2870	2928, 2862, 2322	2928, 2866	2928, 2862, 2322	2932, 2866, 2318	2932	2939, 2326
C=C _{str}	1616	1616	1639	1639	*	*	1643	1632
C=C-C _{str} Aromatic	1520, 1447	1520, 1447	1535, 1404	1531, 1447	1531, 1450	1524, 1447	1408	1408
C-O _{str} aromatic	1366	1362, 1269	1246	1265	1373, 1246, 1184	1385, 1246	1254	1234
C-O-C)	1053	1057	1069	1065	1092	1076	1069	1061
C=O	*	*	*	1717	1724	1701	*	*

Key: REE- root ethanolic extract; SEE-stem ethanolic extract; LEE- leaves ethanolic extract; PEE-pods ethanolic extract; F^A-LEE fraction 1; F^B-LEE fraction 2; F^C-LEE fraction 3; F^D-LEE fraction 4.

Table: 4.Acute toxicity study of *P. juliflora* LEE after oral administration to rats

Dose (mg/kg) body weight	Sex				Total number of rats used
	Male		Female		
	O (survived)	X (died)	O(survived)	X (died)	
175	1	0	1	0	2
550	1	0	1	0	2
1750	2	1	2	1	6
2000	3	0	3	0	6
5000	3	0	3	0	6

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