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CHEMICAL COMPOSITION, ANTIMICROBIAL AND CYTOTOXIC ACTIVITIES OF ESSENTIAL OIL AND LIPOIDAL MATTER OF THE FLOWERS AND PODS OF *TIPUANA TIPU* GROWING IN EGYPT

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ABSTRACT

Hydrodistillation of the flowers of *Tipuana tipu* yielded 0.5% v/w of oil. Eighteen components were identified by GC/MS analysis and Perhydro-farnesyl (32.17%) was the major constituent. Qualitative a and quantitative analysis of fatty acids and unsaponifiable matter using GC/MS revealed the presence of fifteen fatty acids in leaves and fourteen in pods. Decosane was the major identified hydrocarbon representing (17.6%) in leaves and (12.72%) in pods. .The leaf extract and volatile oil of flowers showed a broad spectrum antimicrobial effect, while pods extract showed obvious activity on *Escherichia coli* (gram –ve bacterium) and yeast. The minimum inhibitory concentrations of the methanolic and chloroformic extracts of leaves and pods were ranged from 0.2-0.6 mg/ml while volatile oil of flowers was 0.05. Leaf extract was found to be stable at 37ºC for 25 h and the remaining activity was 72% to 86%. The activity against all tested microorganisms was completely lost after incubation at 50ºC for 15 h. Storage at room temperature (25ºC) for three months in air tight glass bottle retains the activity. The exposure of the isolated fractions to different pH (2 to 12) for 15 min incubation period showed that fractions retain about 52% to 74% of the activity at pH 2 and 45% to 65% at pH 8 and a complete loss in activity was observed at pH 12. The methanolic extract showed an obvious cytotoxic activity against breast and colon carcinoma cell lines, while the chloroformic extract showed cytotoxic activity against breast and cervix carcinoma cell lines. The volatile oil showed significant cytotoxic activity against the three examined cell lines.

Keywords: *Tipuana tipu,* fabaceae, volatile oil, lipoidal constituents, antimicrobial activity, cytotoxic activity.

INTRODUCTION

Globally Family Fabaceae (Leguminosae) is the 3rd largest family of flowering plants, comprising the three subfamilies: Mimosoideae, Caesalpinioideae and Papilionoideae with nearly 20000 species (Hickey *et al.*, 1997; Lewis *et al.,* 2005). The genus *Tipuana* belongs to the subfamily Papilionoideae and comprises only one species *Tipuana tipu* (Hickey and King, 1997). The plant is beautiful flowering trees with yellow flowers, and it is called pride of Bolivia and yellow jacaranda. It is an indigenous to South America, Southern Bolivia and Northern Argentina, and has been planted allover the world as an ornamental street and garden tree. Genus *Machaerium* is used in South America by indigenous population to treat diarhea (Heinrich *et al.*, 1992) menstruation cramps (Ginzbarg, 1977), coughs (Joly *et al*., 1987) and aphtous ulcers of the mouth. Certain *Machaerium* species were reported to have antimicrobial (Waage *et al*., 1984), antigiardial (Elsohly *et al.*, 1999) and antitumor activities (Seo *et al.,* 2001) with the view of developing new antitumor and antimicrobial substances with low toxic potential, nature-derived compounds still play a major role as drugs and as lead structures for the development of synthetic molecules. Screening for new drugs in plants implies the screening of extracts for the presence of novel compounds and evaluation of their bioactivities. Nothing was reported in the available literature concerning the volatile constituents of the flowers of *Tipuana tipu*, as well as lipoidal matter of the leaves and pods. This directed the present work to carry out a comparative study of the lipid content of the leaves and pods, as well as to identify the constituents of volatile oil of fresh flowers and evaluate their antimicrobial and cytotoxic activities.

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MATERIALS AND METHODS

Plant material

Fresh leaves, flowers and pods of *Tipuana tipu* L (Family Fabaceae) were collected from trees growing in Cairo, Egypt. The flowers were collected in the month of May, while leaves and pods in July [The plant was identified by Mrs. Traes Labib, Plant Taxonomist at El-Orman Botanical Garden, Giza, Egypt. A voucher specimen was deposited in Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University].

Microorganisms for Antimicrobial activity

Gram+ve bacteria: *staphylococcus aureus, Bacillus subtilis*, *Bacillus cerrus,* Gram –ve bacteria: *Escherichia coli.* Fungi: *Aspergillus niger*, *A. flavus, A.terru,* and Yeast: *Saccharomyces carle*s, *Candida albicans, Candida*

pseudotropicales. Microorcanisms were obtained from MIRCEN, Cairo, Faculty of Agriculture, Ein-Shams University, Egypt.

Cell lines for cytotoxic activity (Skehan *et al.*, 1990)
HELA (cervix carcinoma cell line), MCF7 (breast carcinoma cell line), HCT116 (colon carcinoma cell line), 96 multiwell plate (10^4cells/well), Sulphorhodamine B stain, and Tris-EDTA buffer were used.

APPARATUS
GLC: GLC/PYE UNICAM PRO-GC with SP-2300 column for saponifiable matter coupled to an injector temperature 250ºC 1.5 X 4mm**.** GLC/PYE UNICAM PRO-GC with column of methyl phenyl silicone OV-17 1.5x4mm (Faculty of Agriculture, University of Cairo)**.**

Preparation of plant extracts
The powder (200g) of each of the leaves and pods of *Tipuana tipu* was exhaustively extracted by repeated refluxing with light petroleum ether (60-80), chloroform and methanol, in succession. The solvent-free extracts were stored in the dark at -20ºC for further studies.

Preparation of essential oil
Volatile oil of flowers was obtained by steam distillation of fresh plant material in Clevenger type apparatus for 5hrs, affording a pale yellow oil which was dried over anhydrous sodium sulphate. Percentage yield and physical constants were determined according to Egyptian Pharmacopoeia.

GC/MS analysis of essential oil
Volatile oil was dissolved in dichloromethane; GC/MS analysis of was performed with GC/MS (Shimadzu GC/MS-QP5050A), operated in the electron impact mode. Column, capillary DBI, 25m x 0.53 mm ID, 1.5 nm film. Temperature-programming: 40-160ºC at 7.5ºC/min., 160-230ºC at 2.5ºC/min. till 250 ºC. Injector temperature: 250ºC, Detector: FID, maintained at 280ºC; Carrier gas: He, flow-rate of 10 ml/min. Identification of volatile components was achieved by comparison of their retention times and mass fragmentation pattern with published data Adams (1989), Ardakani *et al*. (2003), Stenhagen *et al*. (1970), McLafferty *et al*. (1993), as well as Wikkey 229 LIB data base. Relative percentages were determined by integration of peak areas. Results are presented in table 1.

Preparation and saponification of lipoidal matter
Air-dried powdered leaves and pods (200g) were separately, extracted with light petroleum ether (60-80) till exhaustion. The extracts were evaporated under reduced pressure at temperature not exceeding 40ºC. One gram of each of the extracts was saponified by refluxing with alcoholic potash (10%) for 2 hours (Paech, 1955; Yamaguchi, 1970; Vogel, 1961). After evaporation of the solvent and dilution with cold water, the unsaponifiable

Table 1. GC/MS analysis of essential oils of *Tipuana tipu* flowers.

Peak No.	RRt*	%	M^+	Base peak	(M/Z%)	Constituents
1	0.312	0.58	136	93.10	41,77,93	α-thujene
2	0.325	0.81	226	57.10	43, 57, 69, 108	Heptane
3	0.334	1.34	178	81.10	41,67, 81, 93, 138	Unidentified
4	0.347	0.52	136	93.10	41, 77, 93, 136	α-Phellandrene
5	0.355	0.67	90	119.15	41, 65, 77, 91, 119, 134	p-Cymene
6	0.361	12.47	136	68.10	41, 67, 93, 107, 121, 136	Limonene
7	0.39	5.82	154	41.10	41, 55, 71, 81, 93, 96, 121, 136	β- linalool
8	0.45	0.75	154	59.10	43, 59, 81, 93, 107, 121, 136	α-Terpineol
9	0.482	1.31	154	69.15	41, 68, 69, 93, 123	Trans geraniol
10	0.563	1.25	172	73.10	41, 60, 73, 129	Decanoic acid
11	0.635	3.95	194	43.05	43, 67, 69, 93, 107, 121, 136, 151	Geranyl acetone
12	0.642	1.39	204	41.10	41, 67, 93, 107, 121, 133	α-Farnescene
13	0.661	15.68	226	57.15	43, 57, 71, 85, 99, 113, 127, 141, 183	Hexadecane
14	0.84	1.04	154	43.10	43, 57, 71, 83	Camphene hydrate
15	0.917	1.67	148	105.10	41, 60, 77, 91, 91, 105, 129, 212	Cumin aldehyde
16	1	32.17	226	43.10	43, 58, 71, 85, 109, 124, 149, 165, 210	Perhydro-farnesyl acetone
17	1.067	3.33	270	74.10	43, 55, 74, 87, 143, 227	Methyl palmitate
18	1.104	13.62	256	41.10	41, 55, 73, 83, 97, 115, 129, 157, 213	Palmitic acid
19	1.198	1.61	220	67.15	41, 67, 79, 95, 108	Caryophyllene oxide

RRt: Relative retention time to perhydrofarnesyl acetone with Rt=43.218
M^+: Molecular ion peak
M/z %: mass/charge arranged according to their relative intensities in a descending order.

fraction was extracted with successive portions of diethyl ether (3x15ml) and the combined ethereal extract was washed with distilled water, dried over anhydrous sodium sulphate and filtered was under reduced pressure. The residue left after evaporation of the solvent was weighed and kept for further investigation of USM. The aqueous alkaline solution left after separation of USM in each case was acidified with dilute hydrochloric acid; the liberated fatty acids (FA) being extracted with ether (3x15ml) till exhaustion. The combined ethereal extracts were washed with water till free from acidity and dehydrated over anhydrous sodium sulphate. An aliquot of the fatty acids fraction (0.5g) of each of the two organs was subjected to methylation, evaporated under reduced pressure till dryness and kept in a desiccator for GLC analysis. The percentages of USM and total FA are listed in table 2.

GLC of USM

GLC/PYE UNICAM Pro-GC; column: OV-17 (methyl phenyl silicone); 1.5mx4mm; detector: FID, temperature 300ºC; injector temperature, 250ºC; temperature-programming, 70-270ºC at 10º/min.; carrier gas, nitrogen, flow-rate 30 ml/min; chart speed, 0.4 cm/min. The results are presented in table 3.

GLC of Fatty acids Methyl Esters (FAME)

GLC/PYE UNICAM Pro-GC; column: SP-2300, 1.5mx4mm; detector, FID, temperature, 300ºC; injector temperature, 250ºC; temperature programming: 70-190ºC at 8º/min.; carrier gas, nitrogen, flow-rate: 30ml/min; chart speed, 0.4cm/min. The results are presented in table 4.

Table 2. Percentage yield of lipoidal matter in leaves and pods of *Tipuana tipu* and percentage composition of unsaponifiable matter and total fatty acids.

Plant part	% lipoidal matter	% composition	
		Unsaponifiable matter	Fatty acids
Leaves	0.1	93%	7%
pods	0.2	40%	60%

Table 3. GLC analysis of unsaponifiable matter of *Tipuana tipu* leaves and pods.

Authentic	Carbon No.	RRt*	Relative %	
			Leaves	Pods
n-Tridecane	C13	0.315	0.797	0.2
n-Tetradecane	C14	0.445	1.003	2.050
n-Pentadecane	C15	0.54	1.522	2.294
n-Hexadecane	C16	0.63	4.067	1.067
n-Heptadecane	C17	0.6959	5.861	0.047
n-Octadecane	C18	0.76	0.226	0.358
n-Nonadecane	C19	0.839	0.786	2.912
n-Eicosane	C20	0.89	5.113	3.893
n-Heneicosane	C21	0.93	0.413	1.545
n-Decosane	C22	1.00	17.665	12.721
n-Tricosane	C 23	1.04	0.894	1.901
n-Tetracosane	C24	1.09	0.957	1.124
n-Pentacosane	C25	1.146	1.964	3.121
n-Hexacosane	C26	1.194	2.791	5.807
n-Heptacosane	C27	1.24	4.217	5.899
n-Octacosane	C28	1.295	8.194	7.323
n-Nonacosane	C29	1.36	10.890	8.923
n-Triacontane	C30	1.4	2.619	7.114
Sterols				
Cholesterol		1.6	7.654	2.297
Stigmasterol		2.2	-	1.817
β-Sitosterol		2.3	0.861	12.023
β-amyrin		2.4	1.421	1.007
Percentage of identified hydrocarbons			69.979	68.299
Percentage of identified sterols			9.936	17.144

*RRt: Relative retention time to decosane with Rt=18.95 min.

Antimicrobial Assay

The antimicrobial activities of methanolic and chloroformic extracts of leaves and pods of *Tipuana tipu* and volatile oil from flowers were determined by the agar diffusion technique (Bauer *et al*., 1966; Wilkins *et al*., 1972). Sterile nutrient, malt extract and Czapek's dox agar media were inoculated, separately, with 100µl cell suspension of the tested bacteria, yeast and fungi and poured into Petri-dishes (15cm diameter). 2mg of each of the extracts or fractions was dissolved in 1 ml methanol; a concentration of 0.2 mg was chosen and was placed on filter paper disc (1cm diameter). Solvent was allowed to evaporate and the discs were deposited on the surface of inoculated agar plates and kept at low temperature before incubation which favors diffusion over microbial growth to detect the inhibition zone clearly. The plates were incubated at 35ºC for bacteria and at 30ºC for yeast and fungi. Tetracyclin and metronidazol were used as positive controls for bacteria and fungi, respectively. The antimicrobial activity was expressed as the diameter of inhibition zone in mm. For essential oil, the agar overlay technique using holes or cylinders as reservoir that contact with the inoculated medium was used; oil being diluted with diethyl ether at a concentration of 0.3% (v/v). The results are presented in table 5.

The minimum inhibitory concentration (MIC)

MIC of extracts from leaves and pods, as well as the volatile oil were determined using serial dilutions technique. Different concentrations ranging from 0.2 to 0.6 mg/ml for leaves and pods extracts and a concentration of 0.05 to 0.3% (v/v) of volatile oil were prepared and tested against the chosen microorganisms. Experiments were performed in duplicate and diameters of inhibition zones were measured. MIC was expressed as the lowest concentration of plant extract that produced complete growth inhibition and the results are presented in table 6.

Thermostability of *Tipuana tipu* leaf extract

This was determined at 37ºC and 50ºC. Samples were pre-incubated for 5, 10, 15, 20 and 25hours at the appropriate temperature, followed by rapid cooling. The autoclaved sample was also bioassayed. The most sensitive strain of each group (Gram positive and Gram negative bacteria, yeast and fungi) was chosen as a representative organism. The remaining antimicrobial activity was estimated by the agar diffusion method (Dixon *et al*., 1983). Evaluation of the stability of leaf extract of *Tipuana tipu* at different pH values was carried out. The samples were incubated at different pH values for 15 min. and 30 min. at 4ºC, prior to evaluation of the remaining activity by the agar diffusion method. The results are shown in figures 1-4.

Evaluation of cytotoxicity (Skehan *et al*., 1990)

Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 hrs before treatment with the compounds to allow attachment of cells to the wall of the plate. Different concentrations of the compound under test (25-100µl/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound for 48hrs at 37ºC in an atmosphere of 5% CO_2. After 48hours, cells were fixed with trichloroacetic acid (TCA), washed and stained with Sulphorhodamine B (SRB). Excess stain was washed with acetic acid and attached stain was recovered with Tris-

Table 4. GLC analysis of FAME of leaves and pods of *Tipuana tipu*.

Authentic of FAME	Carbon No	RRt*		Relative %	
		Leaves	Pods	Leaves	Pods
Caprylic	C8:0	0.329	0.248	0.506	0.531
Pelargonic acid	C9:0	0.437	0.312	0.138	0.039
Capric acid	C10:0	0.602	-	0.382	-
Lauric acid	C12:0	0.684	0.502	0.607	0.148
Lauroleic	C12:1	0.733	0.521	0.121	0.174
Myristic acid	C14:0	0.843	0.619	6.448	0.938
Myristoleic acid	C14:1	0.869	0.658	25.053	1.625
Pentadecanoic acid	C15:0	0.93	0.682	1.903	1.004
Pentadecenoic acid	C15:1	0.955	0.704	0.666	0.725
Palmitic acid	C16:0	1.0	0.734	27.262	20.396
Heptadecanoic acid	C17:0	1.125	0.830	13.954	1.078
Stearic acid	C18:0	1.22	0.907	11.892	6.848
Oleic acid	C18:1	1.29	0.933	0.531	11.743
Linoleic acid	C18:2	-	1.0	-	52.046
Linolenic acid	C18:3	1.45	-	0.678	-
Arachidic acid	C20:0	1.63	1.22	4.771	0.286
Percentage of total identified saturated FAME				67.863	31.268
Percentage of total identified unsaturated FAME				27.949	66.313

RRt*: relative retention time of palmitic acid (for leaves) =1, with Rt=17.53. In pods relative retention time of linoleic acid =1, with Rt=22.483.

Table 5. Antimicrobial activity of *Tipuana tipu* leaves, pods and volatile oil of flowers.

Microorganism	*Inhibition zone diameters (mm)						
	**Leaves		**Pods		***Flowers, volatile oil	Positive control	
	MeOH	$CHCl_3$	MeOH	$CHCl_3$		****Tetra-cycline	****Metro-nidazol
Gram positive bacteria							
Staphylococcus aureus	16	16	-	-	23	12	12
Bacillus cerrrus	-	-	-	20	28	30	-
Gram negative bacteria							
Escherichia coli	12	12	12	30	28	30	30
Yeast							
Candida albicans	12	-	12	15	26	30	30
C. pseudotropicales	-	-	12	12	24	35	14
Sacchoromyces carles	20	12	12	20	25	-	-
Fungi							
Aspergillus niger	12	12	12	-	26	12	12
A.. flavus	12	12	-	12	28	-	12
A. terrus	12	12	12	-	24	24	12

*Average of three determinations, **conc. of extract=0.6 mg/ml, ***volatile oil =0.3%, ****conc. of antibiotic=0.05 mg/ 0.1 ml in each disc.
(-): inactive, 12-14: partially active, 15-20: very active.

EDTA buffer. The absorbance of the stain was read in a VMax microplate reader (ELISA reader) at 564nm. The relation between surviving fraction and drug concentration was plotted to get the survival curve of each tumor cell line after the addition of the specified compound and the results are presented in table 7.

RESULTS AND DISCUSSION

Hydrodistillation of the flowers of *Tipuana tipu* cultivated in Egypt yielded 0.5% v/w of a pale yellow oil having a pale yellow colour and a faint characteristic odour. The oil has a specific gravity of 0.85 and a refractive index of 1.508. GC-MS analysis of the oil allowed the identification of 18 components presented in table 1. The oxygenated components were found to be the main constituents (69.16%) while the percentage of hydrocarbons amounted to (29.48%). Monoterpene hydrocarbons were found mainly limonene (12.4%) and α-phellandrene (0.52%); while α-farnescene (1.39%) being the main sesquiterpene hydrocarbon. Major oxygenated monoterpenes were β- linalool (5.82%) and geranyl acetone (3.95%); while caryophyllene oxide (1.61%) being the main oxygenated sesquiterpene. The major constituent of the oil (32.17%) was pentadecanone (perhydrofarnesyl acetone). Fatty acids and esters exhibited (17.84%) of the volatile constituents, of which palmitic acid represented (13.26%). The percentage of FA is higher in pods (60%) than in leaves (7%); while the percentage of USM is higher in leaves (93%) than in pods. GLC analysis of USM indicated that the percentage of identified hydrocarbons in leaves and pods was 69.975 % and 68.299%, respectively. The major hydrocarbon in leaves and pods was Decosane (17.6%) in leaves and (12.72%) in pods. The major hydrocarbons in leaves were n-nonacosane C_{29} (10.89%), n-octacosane (8.194%), n-eicosane C_{20} (5.43%), n-heptacosane C_{27} (4.217%), n-heptadecane C_{17} (5.861%), n-hexacosane C_{26} (2.8%) and n-pentacosane C_{25} (1.96%), while in pods the major hydrocarbons were n-nonacosane C_{29} (8.923%), n-octacosane C_{28} (7.323%), triacontane C_{30} (7.114%), n-hexacosane C_{26} (5.8%) n-heptacosane C_{27} (5.899%), n-eicosane C_{20} (3.89%) , n-pentacosane C_{25} (3.121%) and n-tetradecane C_{14} (2.05%). GLC revealed that β-sitosterol, Cholesterol and β-amyrin were the major sterol contents in leaves in addition to stigmasterol in pods.Campesterol and avensterol were previously reported from the seed of *Tipuana tipu* using GC-MS (Maestri *et al.*, 2002). GLC analysis of the fatty acid methyl ester of the leaves and pods of *Tipuana tipu* showed fifteen fatty acids in leaves and fourteen in pods. The percentage of unsaturated FAME is (27.949%) in leaves and (66.313%) in pods. The major unsaturated FAME in leaves was myristoleic acid (25.053%), while that of pods was linoleic acid (52.046%). The percentage of saturated FAME was (67.863%) in leaves and (31.268%) in pods. Palmitic acid represents the main saturated FAME in both organs with a relative percentage of (27.262%) in leaves and (20.393%) in pods.

Antimicrobial activity

The antimicrobial activities of the leaves, pods extracts and volatile oil of flowers of *Tipuana tipu* were screened using agar diffusion method (Table 5). It is noticed that the leaf

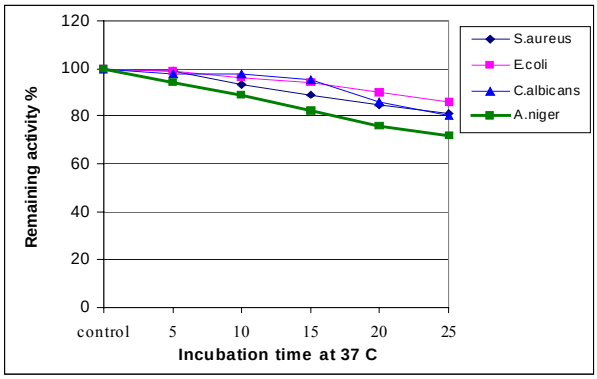

Fig. 1. Effect of different incubation time at 37ºC on antimicrobial activity of *Tipuana tipu* leaves.

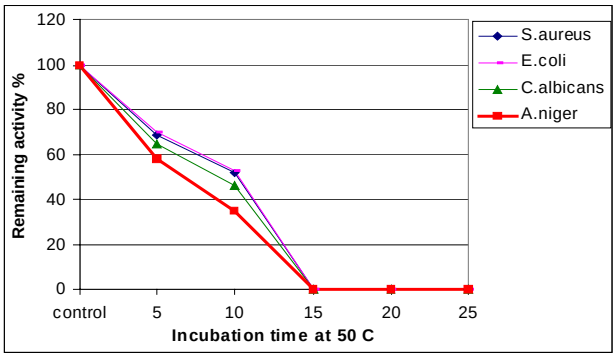

Fig. 2. Effect of different incubation time at 50ºC on antimicrobial activity of *Tipuana tipu* leaves.

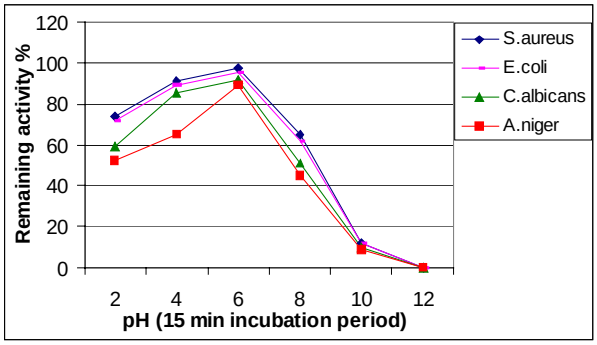

Fig. 3. Effect of different pH on antimicrobial activity of *Tipuana tipu* leaves after 15min incubation period.

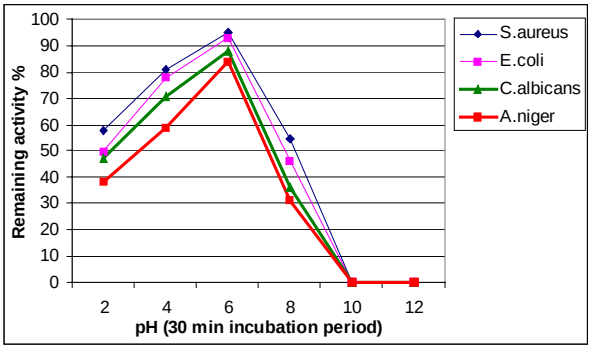

Fig. 4. Effect of different pH on antimicrobial activity of *Tipuana tipu* leaves after 30 min incubation period.

extract and volatile oil of flowers showed a broad spectrum antimicrobial effect while pods extract has more obvious activity on *E. coli* (gram –ve bacterium) and yeast. Regarding the chemical composition of essential oils with respect to the antimicrobial activity (Cimanga *et al*., 2002), Linalool has previously been reported as having antibacterial and antifungal activity (Mazzanti *et al*., 1998), as well as limonene having a higher concentration (12.47%) was active against gram positive and negative bacteria (Pepeljnjak *et al.*, 2005). A recent study reported Perhydrofarnesyl acetone to have moderate antibacterial activity (Yayli *et al.*, 2006; Sanches *et al.*, 2005). Palmitic acid is present at high concentration in the three fractions and is known for its high antibacterial activity (Yff *et al.*, 2002).

The minimum inhibitory concentration

The minimum inhibitory concentrations of the methanolic and chloroformic extract of leaves and pods presented in table 6, were ranged from 0.2-0.6 mg/ml while volatile oil of flowers was 0.05%. This indicates that the isolated fractions have a good activity against microorganisms.

The properties of *Tipuana tipu* leaf extract

The effect of different incubation time at 37ºC on the antimicrobial activity (Fig. 1) shows that leaf extract was stable at 37ºC for 25 h and 72% to 86% of the activity was remained. Incubation at 50ºC showed a noticeable decrease in activity with increasing the incubation time. About 35% to 53% remaining activity was detected after 10h incubation. The activity against all tested microorganisms was completely lost after 15hours of incubation at 50ºC (Fig. 2). When the fraction was autoclaved at 121ºC for 10 min, no antimicrobial activity was retained. Storage at room temperature (25ºC) for three months in air tight glass bottle retains its activity.

The exposure of the isolated fraction to different concentrations of hydrogen ion (pH 2 to 12) for 15 min and 30 min incubation period showed that the incubation for 15 min (Fig. 3) retain about 52% to 74% of the activity at pH 2 and 45% to 65% at pH 8. The remaining activity was about 9% to 12% at pH 10 while a complete loss in activity was observed at pH 12.

Incubation for 30 min. at different pHs showed a noticeable decrease at highly acidic and basic solutions (Fig. 4). The remaining activity was about 38% to 58% at pH 2 and about 31% to 55% at pH 8, while no activity was noticed at pH 10 and 12. Thus the best storage pH range was 4 to 6 at room temperature.

It is to be noticed that the activity of the uncharged form of the phenolic compounds is able to penetrate the microbial cell and exhibit activity (Burkill *et al.*, 1985). So changes in the pH highly affect the activity. These results can serve as a guide in therapeutic drug development having a source of

Table 6. The MIC of methanolic and chloroformic extracts of *Tipuana tipu* leaves, pods and volatile oil of flowers.

Microorganism	Classification	MIC of *Tipuana tipu* extracts mg/ml				
		Leaves		Pods		Volatile oils
		MeOH	$CHCl_3$	MeOH	$CHCl_3$	
Staphylococcus aureus	Gram positive bacterium	0.2	0.2	-	-	0.05%
Escherichia coli	Gram negative bacterium	0.4	0.4	0.6	0.4	0.05%
Candida albicans	Yeast	0.6	-	0.4	0.4	0.05%
Aspergillus niger	Fungi	0.4	0.4	0.6	-	0.05%

%: concentration of the volatile oil (v/v).

Table 7. *In vitro* testing for cytotoxic effect of the methanolic and the chloroformic extracts of *Tipuana tipu* on different cell lines.

Cell line	Sample	* % inhibition of cell viability				**IC_{50}
		10µg/ml	5µg/ml	2.5µg/ml	1µg/ml	
HELA	Methanolic extract	44.6	30.7	27.5	12.3	-ve
	Chloroformic extract	52.6	50.4	44.6	40.4	5.45 µg
	Volatile oil	50	45.8	42.3	40.5	5.3 µg
MCF7	Methanolic extract	57.3	53.6	43.1	12.4	4.30 µg
	Chloroformic extract	61.4	52.4	39.3	34.8	4.56 µg
	Volatile oil	60.2	53.5	44.5	30.6	4.6 µg
HCT116	Methanolic extract	51.3	39.7	30.9	29	9.97 µg
	Chloroformic extract	30.8	27	27.4	13.2	-ve
	Volatile oil	50.3	44.5	40.4	28.3	4.5 µg

*Each result is a mean of three tests
**IC_{50} : dose of the compound which reduces survival to 50%.

new compounds by the selection of some plants as *Tipuana tipu* having antimicrobial activities, and this will represent a non expensive as well as available source. It is probably useful in controlling some plant diseases (Suchiya *et al*., 1996).

Cytotoxic activity

The methanolic extract shows an obvious cytotoxic activity against breast and colon carcinoma cell lines, while the chloroformic extract shows cytotoxic activity against breast and cervix carcinoma cell lines. The volatile oil shows significant cytotoxic activity against the three examined cell lines.

REFERENCES

Adams, RP. 1989. Identification of Essential Oils by Ion Trap Mass Spectroscopy. Academic press Inc., Illinois, USA.

Ardakani, MS., Mosaddegh, M. and Shafaati, A. 2003. Volatile constituents from the aerial part of *Verbena officinalis L.* (Vernain). Iranian. J. pharmaceutical research. 39-43.

Bauer, AW., Kirby, WMM., Sheriss, JC. and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clinic, Path. 45:493-495.

Burkill, HM., Dalziel, JM. and Hutchinson, J. 1985. The useful plants of West Tropical Africa. Royal Botanic Gardens, (2nd edition).

Cimanga, K., Kambu, K., Tona , L., Apers, S., De Bruyne, T., Hermans, N., Totte, J., Pieters, L. and Vlietinck, AJ. 2002. Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. J. Ethnopharmacol. 79 (2):213-219.

Dixon, RA., Dey, PM. and Lamb, CJ. 1983. Phytoalexins: enzymology and molecular biology. Adv. Enzymol. 55:1-7.

Elsohly, HN., Joshi, AS. and Nimrod, AC. 1999. Antigiardial isoflavones from *Machaerium aristulatum*. Plant Med. 65(5):490-494.

Ginzbarg, S. 1977. Plantas medicinales de los indios bribris y cabécar. Am.Indigena. 37:367- 370.

Heinrich, M., Rimpler, H. and Barrera, HNA. 1992. Indigenous phytotherapy of gastrointestinal disorders in a low land mixe community Oaxaca, Mexico. J. Ethnopharm. 36:63-68.

Hickey, M. and King, C. 1997. Common Families of Flowering Plants. International Legume Database and Information Service. Cambridge University.

Joly, LG., Guerra, S., Septimo, R., Soli, SPN., Correa, M., Gupta, M., Levy, S. and Sandberg, F. 1987. Ethnobotanical inventory of medicinal plants used by the Guayami Indians in Western Panama. Part I. J. Ethnopharmacol. 20:145-149.

Lewis, G., Schrire, B., MacKinder, B. and Lock, M (eds.). 2005. Legumes of the world. Royal Botanical Gardens, Kew, UK.

Maestri, DM, Fortunato, RT., Guzman, CA., Torres, MM. and Lamarque, AL. 2002. Seed compositional studies of some species of Papilionoideae (leguminosae) native to Argentina. J. Sci. Food. Agriculture. 82(3):248- 244.

Mazzanti, G., Battinelli, L. and Salvatore, G. 1998. Antimicrobial properties of the linalool-rich essential oil of *Hyssopus officinalis* L. var. *decumbens* (Lamiaceae). Flav. Fragr. J. 13:289- 293.

McLafferty, FW. and Turecek, F. 1993. Interpretation of Mass Spectra, (4th edition). University Science Books, Mill Valley, California, USA.

Paech and Tracey 1955. Modern Methods of Plant Analysis IV. Berlin, Gottingen, Heidelberg.

Pepeljnjak, S., Kosalec, I., Kalodera, Z. and Blazevic, N. 2005. Antimicrobial activity of juniper berry essential oil (*Juniperus communis* L., *Cupressaceae*. Acta. Pharm. 55:417- 423.

Sanches, NR., Cortez, DAG., Schiavini, MS., Nakamura. CV. and Filho, BPD. 2005. An evaluation of antibacterial activities of *Psidium guajava*. Braz. arch. biol. technol. 48(3):429-423.

Seo, EK., Kim, NC., Mi, Q., Chai, H., Wall, ME., Wani, MC., Navarro, HA., Burgess, JP., Graham, JG., Cerbieses, F., Tan, GT., Farnsworth, NR., Pezzuto, JM. and Kinghorn, D. 2001. Two Cytotoxic Compounds from the Stem of *Machaerium aristulatum*. J. Nat. Prod. 64:1483-1488.

Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, JT., Bokesch, H., Kenney, S. and Boyd. MR. 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. J. Natl. Cancer Inst. 82:1107- 1110.

Stenhagen, E., Abrahamsson, S. and McLafferty, FW. 1970. Atlas of Mass Spectral Data. John Wiley and sons, New York, USA.

Suchiya, H., Sato, M., Miyazaki, T., Fujiwara, S., Tanigaki, S., Ohyama, M., Tanaka, T. and Iinuma, M. 1996. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*. J. Ethnopharmacol. 50:27-31.

Vogel, AI. 1961. Practical Organic Chemistry, (3rd edition). Longmans Private LTD, Calcutta, Bombay, Madras, India.

Waage, SK., Hedin, PA. and Grimley, E. 1984. A biologically active procyanidin from *Machaerium floribundum*. Phytochemistry. 23(12):2785-2788.

Wilkins, TD., Holdeman, JJ., Abramson, IJ. and Moore, WEC. 1972. Standardized single-disc method for antibiotic susceptibility testing of anaerobic bacteria. Antimicrob Agents Chemother. I. 1(6):451-455.

Yamaguchi, K. 1970. Spectral Data of Natural Compounds. Elservier Publishing Co., New York, USA.

Yayli, N., Guleç, C., Úçűncű, OS., Yaşar, A., Ulker, S., Coşkunçelebi, K. and Terzioğlu, S. 2006. Composition and antimicrobial activities of volatile components of *Minuartia meyeri*. Turk. J. Chem. 30:71-74.

Yff, BTS., Lindsey, KL., Taylor, MB., Erasmus, DG. and Jager, AK. 2002. Pharmacological screening of *Pentanisia prunelloides* and isolation of the anti-bacterial compound palmitic acid. J. Ethnopharmacol. 79:101-109.