www.ThePharmaJournal.com

The Pharma Innovation

ISSN: 2277- 7695
TPI 2015; 4(4): 90-93

www.thepharmajournal.com
Received: 21-04-2015
Accepted: 18-04-2015

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Chemical composition and antimalarial activity of extracts of Sudanese *Tamarindus indica* L. (Fabaceae)

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Abstract
Tamarindus indica L. (Asteraceae) is a multipurpose tree, either nutritional or medicinal and grows wild throughout Sudan and other parts of the world. The plant fruits are claimed for their antimalarial activity and treatment of fever and gastrointestinal disorders. Crude 96% ethanolic and water extracts in addition to hexane, chloroform, methanol and water successive extracts were prepared to assess their antiplasmodial activity.
The chloroform successive extract exhibited the highest activity (IC_{50}= 34.8 µg/mL) and was subjected for GC-MS analysis. It was composed of aliphatic saturated alcohols (12.15%), aliphatic saturated fatty acids and their derivatives (17.41%) phenolic compounds (2.67%); aromatic acids (0.58%); γ-sitosterol (2.33%) and the most dominant compound 5-hydroxymethyl furfural (30.74%). The activity may be due to one or a combination of these constituents and further studies should be done to spot more light on the observed antimalarial activity and chemical composition of the chloroform extract.

Keywords: *Tamarindus indica*, fruits, antimalarial activity, chloroform extract, 5-hydroxymethyl furfural.

1. Introduction
Tamarind or *Tamarindus indica* L. (Fabaceae) is a multipurpose tree of which almost every part finds at least some use, either nutritional or medicinal (1). The tree grows wild throughout Sudan and is used traditionally to treat many diseases in addition to its nutritional value. African countries do not produce tamarind on a commercial scale and the major production areas are in Asia and South America (2). Tamarind is used in African and Asian traditional medicine in treatment of many diseases such as fever, dysentery, jaundice and gastrointestinal disorders (3, 4). The tamarind fruit has mainly pulp and seeds, the pulp constitutes 30-40% of ripe fruit, seeds around 25-40%; shell and fibre account for 11-30% (5). The most outstanding characteristic of tamarind is its sweet acidic taste mostly due to tartaric acid (8-18%), total carbohydrates (56- 82%) and reducing sugars (25-45%). The pulp is rich in minerals and volatile constituents which include furan derivatives (44%) and carboxylic acids (19%).
Claims have been reported about uses of tamarind in Sudanese traditional medicine as a herbal infusion for malaria fever. To evaluate the scientific bases for the use of the plant in treatment of malaria fever, extracts of different polarities were assessed against *Plasmodium falciparum* for antimalarial activity. A number of research articles and reviews discussing naturally occurring antimalarial agents have been published with examples of alkaloids (6), terpenoids (7); saponins (8); flavonoids (9), coumarins (10), naphthoquinones (11), anthraquinones and xanthonones (12).
The results of assessment of antimalarial activity of *T.indica* pulp extracts and investigation of the chemical composition of the fruit pulp are reported in the present work.

2. Materials and Methods
Plant material
Fruits (pods) of *T.indica* were collected from trees growing in Khartoum district and authenticated at the department of Botany, Faculty of science, University of Khartoum, Sudan.

Extraction of Plant Material
The pulp was removed from the pods and chopped for extraction with 96% ethanol and with distilled water. The first sample (100g) was extracted in a Soxhlet apparatus for 8 hours and the solvent was removed under reduced pressure to yield about 80g extract (79.42%). Another 100g was extracted with distilled water at room temperature for 24 hrs followed by removal

and filtration of extract. The procedure was repeated until a clear colourless extract was obtained. The bulk extract was concentrated and freeze-dried to give about 45g extract (45.85%).

A third sample (100g) was successively extracted in a Soxhlet apparatus with hexane, chloroform and methanol to yield 0.05g; 0.20g and 70.40g respectively. The marc after drying was extracted with distilled water at room temperature and extracts were concentrated and freeze- dried to yield 4.60g.

Assessment of antimalarial activity of the pulp extracts

The prepared crude extracts with 96% ethanol and distilled water and the successive extracts prepared with hexane, chloroform, methanol and water were dissolved in DMSO (dimethyl sulfoxide) and quantitatively assessed by 50% inhibitory concentration (IC_{50}) against *Plasmodium falciparum* according to the jar method (13). Results were assessed according to data published in current literature (14, 15).

Chromatographic analysis by Gas chromatography and Mass spectrometry (GC-MS)

The chloroform active fraction was investigated by thin layer chromatography (TLC) and Gas chromatography mass spectrometry analysis (GC-MS). Analysis of the sample was performed on a QP-2010 (Shimadzu) gas chromatograph coupled with mass spectrometer. The sample was injected into Rtx-5 MS capillary column (60 m length x0.25 mm internal diameter and 0.25µm film thickness. Carrier gas was helium at a flow rate 1.0 ml /min. and linear velocity 25.6 cm/sec. Initial column temperature was 80°C with linear increase 10°C/min up to 280°C. Injection port temperature was 280°C and sample volum was 1µL. The mass spectra were recorded in EI mode at 70 eV. Identification was based on NIST spectral Library data NIST version 1.10 beta, Shimadzu.

3. Results and Discussion

The fruit pulp of *T.indica* was treated with the universal solvent 96% ethanol to extract most of the components in the sample to monitor the claimed antimalarial activity. Distilled water was used as a recommended solvent for extraction based on traditional use of the drug, and successive extraction with hexane, chloroform, methanol and water of the pulp was used to monitor the claimed activity based on polarity of solvents (table 1)

Table 1: Results of crude and successive extractions of *T.indica* fruit pulp.

Solvent	Yield % (w/w)
1. 96 % Ethanol	79.42
2. Distilled Water	45.85
3. Hexane	0.05
4. Chloroform	0.20
5. Methanol	70.40
6. Distilled water	4.60

The prepared extracts were dissolved in DMSO and screened for antiplasmodial activity according to the jar method by Trager and Jensen (13). Results were assessed according to data published in the current literature about quantitative assessment by 50% inhibitory concentration (IC_{50}) against *P. falciparum*: significant activity if IC_{50} < 10 µg/mL; moderate activity if IC_{50} = 10-50 µg/mL and insignificant activity if IC_{50}> 100 µg/mL (14, 15). According to that classification, the hexane, methanol and water successive extracts were inactive with IC_{50}= 191.8; 222.7 and 150.6, respectively. The crude water extract gave IC_{50}=94.4 and the chloroform successive extract gave IC_{50} = 34.8 µg/mL and were assessed as low and moderate activities, respectively (table 2).

Table 2: Results of assessments of IC_{50} of extracts of *T.indica* fruit pulp.

Type of Extract	IC_{50} (µg/mL)
1. 96 % Ethanol	186.9
2. Distilled Water	94.4
3. Hexane	191.8
4. Chloroform	34.8
5. Methanol	222.7
6. Distilled water	150.6

Based on these results, the chloroform successive extract showed moderate activity against *P.falciparum*, which was the highest among the tested extracts and was subjected for further investigation by gas chromatography mass spectrometry analysis to spot more light on its qualitative and quantitative composition (Fig. 1 and Table. 3).

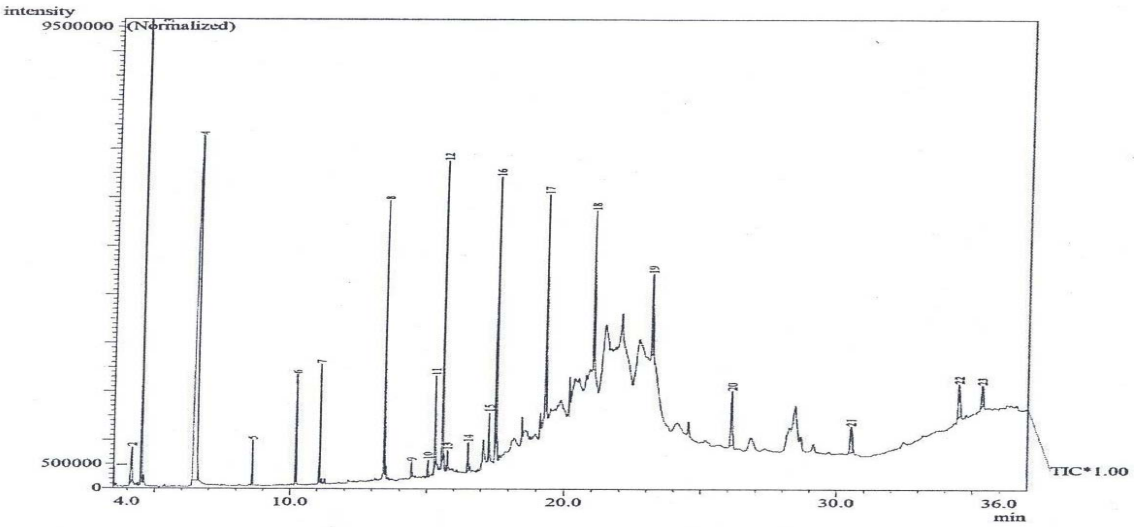


Fig 1: Gas chromatogram of the active chloroform successive extract of *T. indica* fruit pulp.

Table 3: Results of GC-MS analysis of the active chloroform successive extract of *T.indica* fruit pulp

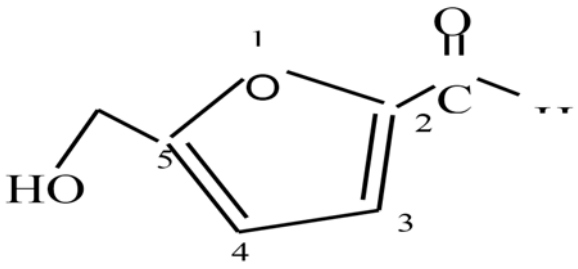
Peak Number	Retention time	%	Base Peak (m/e)	Name
1.	3.52	0.40	57	Decane
2.	4.16	1.55	43	4-oxo-pentanoic acid
3.	4.51	11.63	57	2-Butoxyethyl acetate
4.	6.55	30.74	97	5-Hydroxymethyl-2-furancarboxyaldehyde
5.	8.60	0.98	57	n-Tetradecane
6.	10.20	2.67	191	2,4-bis[1,1-dimethylethyl] phenol
7.	11.08	2.56	57	n-Hexadec-1-ene
8.	13.43	5.84	83	n-Nonadecane
9.	14.43	0.48	83	n-Pentadecane
10.	15.03	0.45	57	7,9-Di-tert-butyl-l-oxaspiro (4,5) deca-6, 9- diene-2,8-dione
11.	15.29	2.54	73	Pentadecanoic acid
12.	15.56	7.67	83	Nonadecane
13.	15.75	0.58	263	3,5-Di-tert-butyl-4-hydroxyphenylpropionic acid
14.	16.51	0.90	83	Octadecan-1-ol
15.	17.26	1.24	73	Octadecanoic acid
16.	17.50	6.71	97	Nanadec-l-ene
17.	19.29	6.63	97	n-Tetracosan-l-ol
18.	21.08	5.29	97	n- Eicos-l-ene
19.	23.14	3.04	97	n-Triacontane-l-ol
20.	26.10	2.83	97	Unknown
21.	30.51	1.92	57	Unknown
22.	34.48	2.23	329	γ-Sitosterol
23.	35.33	1.10	97	n-Hentetracontan-l-ol

The gas chromatogram showed the presence of 23 components of which 21 have been identified. The identified compounds represented about 92.90% of the extract and the unknown compounds about 6.48%. According to the published data in the literature (5,16), tamarind fruit pulp and seed contains volatile and non- volatile compounds of which aromatic and furan derivatives were dominant, and the composition of the pods varies with aromatic conditions. Tartaric acid is the major organic compound among the non-volatiles along with the major constituents, 2-phenyl acetaldehyde, furfural, hexadecanoic acid and limonene. Based on our results, the successive chloroform extract of the Sudanese *T.indica* fruit pulp was composed of aliphatic hydrocarbons (Saturated, 14.15% and unsaturated 14.56%); aliphatic saturated alcohols (12.15%); aliphatic saturated acids (3.78%); aliphatic keto acid (1.55%); aliphatic esters (11.63%); lactones (0.455%); aromatic phenols (2.67%); aromatic acids (0.58%), heterocyclic aldehydes (30.74%) and sterols (2.33%) (Table.4).

Table 4: Chemical composition of the active chloroform successive extract of *T. indica* fruit pulp.

Compounds	%
A. Aliphatic Hydrocarbons Saturated Unsaturated	14.89 14.56
B. Alcohols Aliphatic, Saturated	12.15
C. Fatty Acids Aliphatic, Saturated Aliphatic, ketone Aliphatic, Ester	03.78 01.55 11.63
D. Lactones Spiro-	0.45
E. Aromatic Phenols	02.67
F. Aromatic Acids	0.58
G. Heterocyclic Aldehydes	30.74
H. Sterols	02.33
I. Unknowns	04.15

The main component of the chloroform extract was 5-ydroxymethyl furfural (HMF), 30.74%. It is an organic compound derived from, dehydration of certain sugars and practically absent in fresh food, but naturally generated in Sugar-containing food during heat- treatments like drying or cooking. It is also slowly generated during storage and higher quantities are found naturally in coffee and dried food (17).



5- Hydroxymethyl furfural

4. Conclusion

The claimed antimalarial activity of *T.indica* fruit was assessed against *Plasmodium falciparum* by extraction of fruit pulp with solvents of different polarities. The chloroform successive extract exhibited the highest activity and was composed mainly of HMF. Aliphatic hydrocarbons, alcohols, acids and their esters, in addition to sitosterol and aromatics were among the identified components. Based on these findings, there are grounds to suggest that the moderate antiplasmodial activity was due to one or a combination of these components. Further investigations should be done to spot more light on the component (s)- activity relationship.

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