Seed-Oil Distillates of Thevetia neriifolia: Analysis and Antibacterial Activity

by

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ABSTRACT

The basis for the ethnomedical use of the seed oils of **Thevetia neriifolia** in topical preparations for burns has been studied.

Seed oils extracted from **Thevetia neriifolia** were distilled and assessed for antibacterial activity. The series of distillate fractions obtained, showed varying inhibition patterns against **Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli** and **Pseudomonas aeruginosa.** On analysis the activity levels of the distillate fractions were found to have a definite correlation with their glc profile. The results point to a potential usefulness of the high temperature (>90°c) seed-oil distillates rather than the untreated seed oils in treating skin lesions.

Aldehydes and volatile acids are among the constituents of the distillates.

INTRODUCTION

Thevetia neriifolia Juss (Fam. Apocynaceae) is a small erect latex — producing plant often growing to a height of 51·6 metres. It thrives well in both arid and wet climates producing flowers and fruits all the year round. The seeds contain up to 70% of fixed oil (Obasi, 1987), and an earlier report indicated that the seed oil has found use in topical preparations for treating infected wounds and burns (Watt and Breyer — Bradwijk, 1962).

The indication that certain vegetable oils tend to acquire antibacterial property when subjected to high temperature treatment has been demonstrated by Mazzetti (1928a,b). However, there has been no report on the assessment of **T. neriifolia** seed oils or seed-oil distillates for anti-infective properties. In this paper we report the antibacterial activities of **T. neriifolia** seed oils and seed-oil distillates against **Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli** and **Pseudomonas aeruginosa.** These organisms are usually associated with infected wounds and burns (Lowbury, 1969; Holder, 1981). An attempt was also made to determine the nature of the constituents in the active distillate fractions by chemical methods and by gas liquid chromatography.

MATERIALS AND METHODS

Preparation of the seed oils and seed-oil distillates

Ripe and unripe seeds of **T. neriifolia** were collected from the main campus of the University of Benin, Benin City, Nigeria. The seeds were dried, ground and extracted with petroleum spirit (60 — 80°) by cold percolation. Removal of the solvent under vacuum produced the seed oils, and 20ml of this was distilled in a 100ml round-bottom flask. The fractions were collected at varying temperature ranges as shown in Tables 1 and 2 and used as such (after cooling) in the antibacterial Test A.

Fractions of the unripe seed-oil distillates which distilled below 90°c were combined and labelled as "distillate x", and those which distilled above 90°c were also combined and labelled as "distillate Y". The combined distillates "X" and "Y" were then further investigated in the antibacterial Test B.

Evaluation of antibacterial activity

The antibacterial evaluation of the seed oils and seed-oil distillates was limited to a qualitative determination of their relative activities against bacteria commonly associated with skin lesions.

Test A

Peptone water culture of the test organisms **Staph. aureus** (NCTC 6571), **E. coli** NCTC 10418), Ps. aeruginosa (NCTC 6749) and clinical strains of **Strept pyogenes** obtained from the University of Benin Teaching Hospital were prepared. 0.5ml of each culture was transferred into eleven sterile cork-fitted tubes resulting in four batches of eleven tubes per batch, each batch representing one of the four different test organisms. 0.05ml of each of the test samples R_1, R_2, R_3, R_4, U_2, U_3, U_4, R_o and U_o (see tables 1, 2 and 3) was seperately added to nine of the culture tubes, and 0.5ml of petroleum spirit (E) was added to the tenth tube. The eleventh tube in each batch was left as such to serve as control (C). The tubes and contents were then incubated at 37°c with continuous shaking for 24 hours. Finally, the contents of each of the forty-four tubes were aseptically inoculated onto nutrient agar plates using an alcohol-dipped and flammed wire-loop. The plates were then incubated at 37°C for another 24 hours, and then examined for the presence or absence of growth of the test organisms.

Test B:

On each of four nutrient agar plates were inoculated 0.5ml of nutrient broth subculture of one of the test organisms. The plates were carefully shaken to homogenise the cultures. Sterile filter papers were aseptically cut into discs of 5mm diameter. The discs were in turn used to soak up 0.5ml of the distillates "X" and "Y" and aseptically transferred onto each of the agar culture plates. Also introduced onto each plate was a third disc soaked in the untreated unripe seed oil. The plates were incubated

at 37°C for 24 hours. Three replicates were carried out. Antibacterial activity was recorded as the width of the clear zone of inhibition surrounding a particular disc.

Gas liquid chromatographic analysis of the seed-oil distillates

1ul sample of 1 in 10 dilutions of the seed-oil distillate fractions in chloroform were chromatographed on a PYE Unicam 304 chromatograph equipped with a flame ionisation detector using a 2m x 4mm ID glass column packed with 5% S.E. 30 on chromosorb W-AW, 100–120 mesh. The operating conditions were injector temperature, 250°C; Detector temperature, 280°C; Column temperature, isothermal at 80°C for 5 mins, then programmed at 10°C rise per minute to 160°C and then isothermal at 160°C for another 5 mins; Carrier gas (nitrogen) flow rate, 50ml per minute.

Identification of Constituents

Standard chemical methods were used for the detection of aldehydes and acids (Atherden, 1969).

RESULTS

Antibacterial activity

Table 3 shows the influence of the seed oils and seed-oil distillate fractions on the growth of the test organisms under conditions of Test A. Distillate fractions obtained from the unripe seed oil were generally found to exhibit a higher degree of inhibition on the growth of the test organisms than distillates from the ripe seed oil. The untreated seed oils (R_0 and U_0) and the hour temperature distilled fraction from the ripe seed oil (R_1) had little or no effect on the growth of the test organisms. The ripe seed-oil distillates (R_2 and R_3) collected between 90 — 100°C showed more inhibitory activity on the growth of the test organisms particularly **Ps. aeruginosa** than fraction R_4 obtained at a higher temperature. On the other hand, the inhibitory activity observed with distillate fractions from the unripe seed oil appeared to increase with the distilling temperatures. Again the most susceptible organism being **Ps. aeruginosa** followed by **E. coli** and **Staph. aureus.** Fraction U_4 which distilled at a temperature of 110°C was found to have clearly inhibited the growth of all the test organisms. The high antibacterial activity observed with the high temperature distilled fractions of the unripe seed oil was further supported by results obtained in Test B as shown in Table 4.

The results show the values of the zones created by the inhibition of the test organisms when challenged with the combined low temperature (<90°c) distilled fractions, 'X' and the combined high temperature (>90°c) distilled fractions, 'Y' from the unripe seed oil. Both fractions exhibited definite inhibitory activities on the growth of all the test organisms examined under the stated conditions, with 'Y' being decidedly more active than 'X'. **Ps. aeruginosa** again appeared to be the most sensitive of the test organisms followed by **Staph. aureus** and **E. coli.** The untreated seed oil showed only slight activity.

Analysis

The seed-oil distillates were analysed by GLC on S.E-30 column. The resulting peaks are shown in Fig. 1. There appeared to be no qualitative difference between the GLC peaks of distillate fractions R_4, R_2 and U_4. The key differences seem to be in the preponderance of peaks between the retention times of 8.5 and 18.5 mins.

Chemical analysis showed that distillate fraction X was readily saponifiable with methanolic potassium hydroxide solution indicating the presence of acidic constituents. On the other hand fraction Y was not appreciably saponifiable under the stated condition. In separate tests with ethanolic solution of ammonia, Schiff's reagent, Tollen's reagent, Fehling's reagent and 2, 4 — dinitrophenylhydrazine, both 'x' and 'y' gave reactions characteristic of the presence of aldehydic constituents.

DISCUSSION

The result presented in table 3 showed that the control (C) and the extracting solvent (E) exhibited no inhibitory activity on the growth of any of the test organisms. Hence any inhibitory activity on the growth of the test organisms can only be attributed to the influence of the test samples.

The untreated seed oils showed little or no inhibitory activities against the test organisms that have been commonly associated with skin lesions especially wonds and burns. On the other hand, some of the seed-oil distillate fractions particularly those that distilled from the unripe seed oil at temperatures higher than 90°C were very effective in inhibiting the growth of the selected test organisms. These observations do raise some doubts as to the effectiveness of the untreated seed oil when used to treat infected wounds and burns as indicated in the report by Watt and Breyer-Bradwijk (1962). There is however a clear indication that the high temperature distilled fraction from the unripe seed oil could be used for practical purposes in the treatment of such skin lesions. The observed antibacterial property of the distillates is also in agreement with the observation of Mazzetti (1928) that high temperature treatment may confer bactericidal properties to certain vegetable oils. This was further supported by results obtained from chemical analysis of the active distillate fractions which indicated the presence of volatile acids and aldehydes. The seed oil has been shown to contain mainly esters of fatty acids (Obasi, 1987). The indication therefore, is that distillation under the stated conditions occasioned definite transformation of the seed oil which may account for the acquired antibacterial property.

The results obtained from the GLC analysis of the seed-oil distillate fractions indicated that the key differences in the active fractions were mainly quantitative (Fig. 1). Fraction U_4 which had the most intense peaks between the retention times of 8.5 and 18.5 minutes was also the most active. It thus becomes highly suggestive that the active constituents in the seed-oil distillate fractions are likely to be among those with retention times between 8.5 and 18.5 minutes. This deduction was fur-

ther illustrated when the relative abundance of constituent peaks from the GLC was plotted against their retention times (Fig. 2). A good correlation was found between the inhibitory activities of the distillates and the relative abundance of constituents whose retention times fall between 8.5 and 18.5 minutes. This correlation could serve as a basis for further attempts at isolating the active constituents.

CONCLUSION

The untreated seed oils of **T. neriifolia** were found to have poor antibacterial activities against organisms commonly associated with skin lesions. This has raised some doubts as to the acclaimed effectiveness of the seed oils in the treatment of burns and wounds. The high temperature distilled fraction from the unripe seed oil was found to exhibit remarkable antibacterial activities against the selected test organisms and could therefore be useful for practical purposes as in topical application for treatment of skin lesions such as infected burns and wounds.

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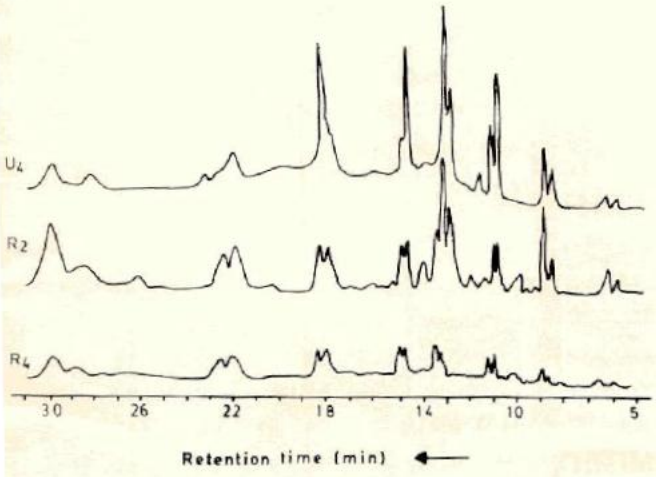


Legend to Fig 1

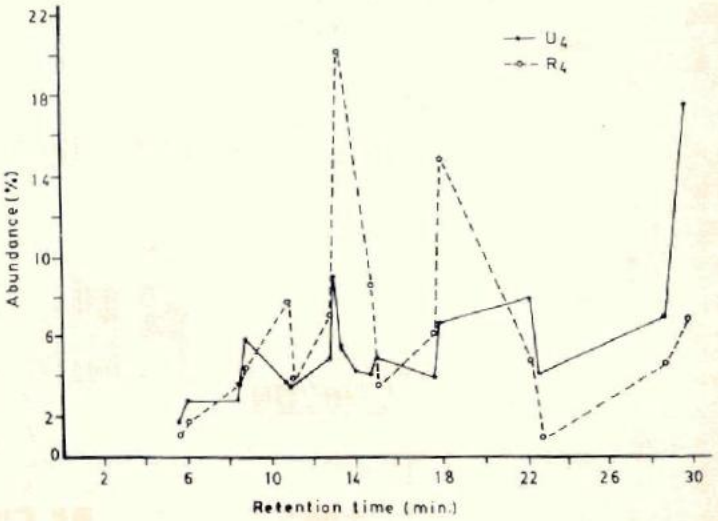
GLC traces for distillates of the seed oils of T. neriifolia

U_4 = Distillate from unripe seed oil (> 100°C)*

R_2 = Distillate from ripe seed oil (90-98°C)*

R_4 = Distillate from ripe seed oil (> 100°C)*

*, distilling temperature



Legend to Fig 2

Plot of percentage abundance against retention times for components of T. neriifolia seed-oil distillates on GLC analysis.

U_4 = Unripe seed oil distillate (> 100°C)*

R_4 = Ripe seed oil distillate (> 100°C)*

*, distilling temperature.

TABLE 1

Distillates from ripe seed - oil (R)

Fraction	(°C)	Volume (ml)	Colour	Other observations
R_1	55 - 58	1.60	Colourless	
R_2	90 - 98	1.60	Light greenish-yellow	Peppery gas evolved
R_3	100	2.20	Darker greenish yellow	
R_4	> 100	2.00	Darker greenish yellow	Burning odour with brown fumes

TABLE 2

Distillates from Unripe Seed-oil (U)

Fraction	(°C)	Volume (ml)	Colour	Other observations
U_1	50	-	-	Gas with choking odour
U_2	65 - 7-	1.00	Light greenish yellow	Odour is characteristic of glacial acetic acid
U_3	98 - 109	1.40	"	Odour is same as U_2 but milder
U_4	> 110	2.60	"	Odour is same as U_3 but milder

TABLE 3

Influence of T. neriifolia seed oils and seed-oil distillates on the viability of different bacteria

Test Sample	Staph. aureus	Strep. pyogenes	E. coli	Ps. aeruginosa
R_0	++	++	++	++
R_1	+++	+++	+++	+++
R_2	+	+	+	NG
R_3	+	+	+	NG
R_4	++	++	++	+
U_o	+	+	+	+
U_2	+	+	+	NG
U_3	+	+	NG	NG
U_4	NG	NG	NG	NG
E	+++	+++	+++	+++
C	+++	+++	+++	+++

R_o, untreated oil from ripe seeds

U_o, untreated oil from unripe seeds

R_1 - R_4, Distillates from ripe seed oil

U_2 - U_4, Distillates from unripe seed oil

E, Extracting solvent (petroleum ether)

C, Control: +++, Heavy growth; ++, light growth:

\+ Scanty growth; NG, No growth.

TABLE 4

Antibacterial activity of unripe seed oil and distillates from T. neriifolia

Diameter of Zone of inhibition (mm)*

Test sample	Staph. aureus	Strept. pyogenes	E. coli	Ps. aeruginosa
X	6.0	6.5	7.0	8.5
Y	10.0	7.5	10.5	12.5
Unripe seed oil	2.5	2.5	2.0	2.0

* Average of 3 determinations

X, Unripe seed-oil distillate obtained below 90°C

Y, Unripe seed-oil distillate obtained above 90°C