Investigation on *Monodora tenuifolia* Seed Oil (Annonaceae)

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Abstract

Fixed oil of Monodora tenuifolia (Family: Annonaceae) a seasonal spice was isolated and the physical and chemical characteristics determined. The physicochemical properties of the oil were comparable to conventional vegetable oils, and the oil contains β-carotene and phospholipids. The extract exhibited remarkable antimicrobial activities against some test organisms: Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Aspergillus niger. Investigation also revealed the presence of alkaloids, flavonoids, tannins, saponins and glycosides.

Introduction

In our earlier study, we reported on the physiochemical properties of a local spice *Monodora myristica* commonly used in Nigeria, and widely cultivated in East and West Indies, Malaysia, Sri Lanka and Africa (Njoku et al, 1996). *Monodora tenuifolia* another member of the Annonaceae family is also found in the forest regions of East Indies, West Indies, Malaysia, Sri Lanka, and Africa, and is very common in the Southern parts of Nigeria (Talaji, 1965). The plant is widely used in ethnomedicine, especially to relieve toothache as well as in the treatment of dysentery (Nielson, 1979). In nutrition, the seed is used as a flavour (Ogutimein et al, 1989). There are equally reports of its use in the cosmetics industries. and the insecticide properties of the essential oil (Adesomuju, et al, 1991; Ogutimein et al, 1989). There are no reports to the best of our knowledge on the fixed oil as well as its phytochemical properties. Hence in the present investigation we report on the physicochemical, phytochemical and antimicrobial properties of the whole seed obtained from different location in Nsukka, Enugu State, Nigeria.

Materials and Methods

Fresh seeds of *Monodora tenuifolia* were collected from different locations in Nsukka area of Enugu State, Nigeria and the identity of the seeds was authenticated by the taxonomical section of the Department of Botany, University of Nigeria, Nsukka. A voucher specimen was deposited in the herbarium of the Department of Pharmacognosy, University of Nigeria, Nsukka, Nigeria.

The collected seeds were sun-dried and then powdered using a corona mill. The power (200 g) was divided into two equal parts. A part 100 g was extracted with petroleum ether (40-60 °C) *in vacuo* using a soxhlet apparatus. The extracted oil was dissolved in ether, washed with warm water, dried over anhydrous sodium sulfate and filtered. The solvent was removed from the filtrate *in vacuo* to leave a light yellow oil. The oil was analysed for sterol using the unsaponifiable fraction as described by Stadam (1957)). β-carotene was estimated also using the unsaponifiable fraction following the method of Bassir (1978). Phospholipid content was determined by extracting the oil with glacial acetic acid followed by the procedure described by Totani et al. (1982). The cyanide content was estimated according to the method of Williams (1979), while gossypol was by the method reported by AOAC (1975). The physicochemical and chemical properties of the oil were also determined by the same AOAC method (1975). The other fraction of the sample 100 g was used to determine the phytochemical constituents of the seed. The chemical tests were carried out on the seed following the methods of Harbone (1984). Tannins were quantitatively assessed by the method of Price and Butter (1977), saponins by the method reported by Achinewhu (1983), and Cyanogenic glycoside by the alkaline picrate method reported by Ikediobi et al (1980).

Antimicrobial studies: Pure clinical culture slants of 5 organisms namely *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Aspergillus niger* were obtained from the medical diagnostic laboratory of the Department of Microbiology, University of Nigeria, Nsukka, Nigeria. Cultures were maintained on nutrient agar slants and preserved at 4 – 5 °C before screening for antimicrobial activity.

The cup plate method was used. Holes of 7 mm diameter were made in the plates of sensitest agar medium with a sterile cork borer. Each plate was spread with a loopful of an over-night culture of the test organism. The holes were filled with 0.4 ml of the *Monodora tenuifolia* oil extract. Controls with brain heart infusion broth were incubated at 37 °C for 18 hr. The zone sizes were measured with calipers. A zone of 10 mm excluding diameter of the cup was taken as an indicator of active inhibition.

Determination of minimum inhibitory concentration (MIC): The method described by Gamod et al (1981) was followed, using brain heart infusion broth and with a starting initial concentration of 25 mg of the oil extract. The broth

cultures of the test organism were diluted to contain approximately 10^9 organisms/ml. 0.1 ml quantities of the diluted culture were inoculated into the test tubes of brain heart infusion broth containing graded concentration of the oil extract 25 – 150 mg/ml. After 18 h incubation at 37 °C, the tubes were examined for growth by the turbidity in comparison with the controls.

Results and Discussion

There is current emphasis on neutraceuticals, especially on plant materials that are relatively abundant and are easily grown. *Monodora tenuifolia* is widely distributed, and are easily cultivated. There are few studies on the industrial and insecticidal properties of this plant (Ogutimein et al, 1989). In an earlier report the essential oil was found to play important roles as a spice and an insecticide (Adesomuju et al, 1991). The present study (table 1) shows that the *Monodora tenuifolia* is a rich source of fixed oil 35.7 %.

Table 1: Physicochemical properties of *Monodora tenuifolia* fixed oil

Determination	Yield (%)
Colour	Golden yellow
State at room temp.	Liquid
Specific gravity	0.8329
Refractive index	1.438
Viscosity mPas	0.039
% yield	35.7
Acid value mg KoH	3.1
Free fatty acid mg KoH	1.6
Peroxide value mEq/L	0.1
Iodine value (Wijs)	136
Saponification value	151
% Unsaponifiable matter	0.5
% Phospholipids	2.5
% β-carotene	1.8
% Cyanide	0.2
% Gassypol	-
% sterol	1.8

This value is comparable to *Monodora myristica* fixed oil another member of the Annonaceae family (Njoku et al, 1996). The phospholipids and sterol levels in this study were equally low, but the phospholipids could find important industrial uses as an emulsifier. The viscosity, refractive index and specific gravity of the oil are comparable to other vegetable oils and in particular the fixed oil of *Monodora myristica*. Studies on the phytochemical test are shown in Table 2. The plant contains such important phytochemical like flavonoids, saponins, tannins, alkaloids, and cardiac glycosides. Of interest also are the results on the very low levels of Cyanogenic glycoside. For many tropical plants, used in nutrition and therapeutics, there are reported cases of death after consumption. Most of the deaths have the classical cyanide intoxication suggesting high levels of cyanide or Cyanogenic glycosides. It is interesting to note that most plants especially common spices used in many nutritional and drug therapy are low in cyanide (Njoku et al, 1997).

Table 2: Phytochemical analysis of the seed of *Monodora tenuifolia*

Tests	Observation	Yield (%)
Alkaloids	+	ND
Anthracene glycoside	+	ND
Cardiac glycoside	+	ND
Cyanogenic glycoside	+	ND
Flavonoids	+	0.7
O & D glycoside	-	ND
Tannin	+	0.32
Saponins	+	0.14
Steroidal glycoside	+	ND
Reducing sugar	+	ND

+ = present; - = absent; ND Not determined

The current results compliment our earlier studies on *Monodora myristica* (Njoku et al, 1996). The presence of tannin in the seed is noteworthy and could explain the browning associated with the seed on exposure to air. It is possible that the polyphenols easily react with molecular oxygen to initiate the browning reaction. This could lead to problems of acceptability especially of food preparation made using the seed for nutritional purposes. The earlier reports of the insecticidal properties of the plant could be attributed to the saponin content as well as the steroidal glycosides and essential oils (Ogutimein et al, 1989). The presence of flavonoids is interesting. Flavonoids and the vitamins present could play important roles as antioxidants, especially in scavenging free oxygen radicals.

This seems to our knowledge the first report on the antimicrobial properties of the fixed oil of *Monodora tenuifolia*. Summary of the antimicrobial studies is shown in tables 3 and 4.

Table 3: Antimicrobial studies of fixed oil of *Monodora tenuifolia* zone of Inhibition in diameter (mm)

Test organism	Inhibition zone Diameter (mm)
Bacillus subtilis	6.0
Escherichia coli	6.0
Pseudomonas aeruginosa	1.5
Staphylococcus aureus	6.0
Aspergillus niger	6.0

Table 4: Minimum Inhibitory Conc. (mg/ml) of fixed oil of *Monodora tenuifolia*

Test organism	MIC % w/v
Bacillus subtilis	0.19
Escherichia coli	0.24
Pseudomonas aeruginosa	0.12
Staphylococcus aureus	0.4
Aspergillus niger	0.38

In this study, *Monodora tenuifolia* fixed oil exhibited marked activities against the test microorganisms. The actual mechanism is not clearly understood, however the phytochemicals, which are present, as well as the fatty acids of the oil might be responsible for the marked activities. Saponins and long chain fatty acids have been reported to have antimicrobial activities (Njoku et al, 1999). Since the iodine value obtained in this study was high, it is possible that

the oil is rich in some long chain unsaturated fatty acids. Preparative thin layer chromatography reveals that the oil has a lot of fatty acids, which could not be resolved (results not shown). The presence of remarkable antimicrobial activity justifies the traditional use of the plant in the treatment of dental decay.

The Monodora plants generally used as spices in Nigeria have a lot of prospects in both nutrition and therapeutics, and could find important roles to play as alternative food materials in human nutrition.

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