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Larvicidal and Anti-Microbial Potentials of *Nymphaea odorata*

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Abstract: *Nymphaea odorata* (Nymphaeaceae) is an old herbal recipe used in the treatment and or management of ocular, skin, gastrointestinal and urino-genital ailments amongst many others. However, its use in malaria control at the larval stage is yet to be investigated. Hence the larvicidal and anti-microbial studies were undertaken. The larvicidal assay determined in terms of percentage mortality showed that the crude leaf extract gave weak larvicidal activity (LA %) of 10 and 20% (at 5% w/v) and 20 and 30% (at 10% w/v) both at 12 and 24 h incubation, respectively. Surprisingly, the crude extract and fractions were inactive against the bacterial and fungal isolates tested. These results in particular render untenable claims in ethno-medicine of the uses of the plant in treating infections especially those of microbial origin.

Key words: Larvicidal, anti-microbial, inactive, *Nymphaea odorata*, Nymphaeaceae

INTRODUCTION

The genus; *Nymphaea* takes its name from the Greek word; *numphe* which means virgin or water nymphe and is reputed for the anti-aphrodisiac activity of its members. *Nymphaea odorata* (water-lily) is known by common names such as fragrant water-lily, American white-lily and Alligator Bonnet amongst many others (Josh and Emily, 2002). It belongs to the family; Nymphaeaceae which consists of six genera and seventy species (Trease and Evans, 1996) and grows in ponds, marshes and sluggish streams (Odey and Kingdersley, 1993).

Water-lily is an old fashioned herbal remedy which when made into a douche is used to treat eye troubles, leucorrhoea, diarrhea, scrofula, inflamed tissues, bronchial, kidney, bladder and prostrate-gland troubles. The leaves and roots are made into poultice to treat wounds, cuts, bruises, ulcers, boils and painful swellings (Harvey and John, 1898). Tea made with the roots makes a good gargle for irritation and or inflammation of mouth and throat. It can also be used to treat coughs, tuberculosis, remove freckles and pimples from the face and skin (George, 2001).

Compounds such as tannins [tannic acids and gallic acids (anti-microbial], alkaloids[nymphaerine and nupharine] and glycosides [cardenolide and myrictrin] which are antiseptic, astringent and demulcent have been reportedly isolated from this plant (Trease and Evans, 1996). In spite of these, the present study was designed with the aim of investigating into the larvicidal activity and also to confirm or otherwise the sensitivity of the plant extract and fractions to selected microbes.

MATERIALS AND METHODS

Collection of Plant

The fresh leaves of *Nymphaea odorata* were collected in the month of November, 2006 from Idim Itak (Stream of Itak) in Itak Atap Ikono Local Government Area of Akwa Ibom State, identified and

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authenticated by R. Nia of the Department of Pharmacognosy and Natural Medicine, University of Uyo, Uyo, Nigeria where voucher specimen NoH53 was deposited.

Chemicals, Micro-Organisms and Media

The chemicals reagents: Butanol, Ethanol, Chloroform and Hexane (all of AnaLaR grade; Aldrich Chemicals Inc, USA) were purchased in Uyo. Silica gel (254GF), Ampiclox and Ketoconazole were obtained from Unique Pharmaceuticals Limited, Lagos, Nigeria. The micro-organisms (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Candida albicans*) clinically isolated from human specimens; urine, faeces, wounds and vaginal swabs were obtained from the Medical Laboratory, University of Uyo Health Center. They were collected in sterile bottles, identified and authenticated by convectional biochemical tests (Gibson and Khoury, 1986; Murray *et al*., 1995) and then refrigerated at 0-5°C at the Pharmaceutical Microbiology and Parasitology Unit, Faculty of Pharmacy, University of Uyo, Uyo, Akwa Ibom State prior to use. Also, Mueller Hinton II Agar (Biotec Laboratory, Ipswich, England), Sabouraud Dextrose Agar (International Diagnostic Group PLC, Lancaster, England) and Nutrient Broth (Oxiod Limited, Basingstroke, England) were used.

Extraction and Processing

The leaves were air-dried and powdered in an electric mill. The resultant ground powder was then extracted with cold 50% aqueous ethanol at room temperature (27±2°C) for 72 h. The filtrate was evaporated to dryness using a rotary evaporator (Buchi CH-920, Laboratorium Technic, Flawk/SG, Switzerland). The dried crude ethanolic extract was subsequently investigated for plant metabolites (alkaloids, tannins, cardiac glycosides, terpenes, anthraquinones and flavonoids) according to laid down phytochemical methods (Harborne, 1984; Sofowora, 1993; Trease and Evans, 1996). Also, the dried crude ethanolic was chromatographed on silica gel (254GF) column and gradient elution using hexane: chloroform: butanol (1:1:1) mixture. Eluates which showed similar TLC profiles under UV (λ 366) were pooled and bulked separately to obtain the hexane, chloroform and butanol fractions were evaporated to dryness and stored in a refrigerator at -4°C prior to the biological tests.

Larvicidal Assay

The Breeding of Larvae of *Anopheles gambiae*

The larvae were bred by keeping outdoor basins of water under growing shrubs near houses for about two weeks. After this period, at least three groups of mosquitoes larvae were identified accurately in a container using classical methods (Sievers *et al*., 1949). *Anopheles gambiae*, *Aedes agypti* and *Culex piper-fatigans* responsible for the transmission of malaria, yellow fever and filariasis, respectively were so identified. The fourth instar larvae of *Anopheles gambiae* were later selected, separated and the species authenticated at the Department of Entomology, Michael Okpara University of Agriculture, Umidike, Abia State, Nigeria before further work.

The method employed for the determination of larvicidal activity was adopted from that described by several researchers (Ojewole *et al*., 2000) and WHO directives on such assay with modifications (WHO, 1970). Thirty *Anopheles gambiae* larvae in their fourth stage were put in recovery cups (250 mL plastic jars) containing 10 mL de-ionized water (pH 7.0) at room temperature (27±2°C). Three (3 mL) volume each of the graded concentrations of the extracts (5 and 10% w/v) were added to 90 mL de-ionized water, mixed thoroughly and then poured into exposure cups (250 mL plastic jars containing larvae food). Each aqueous solution of the extract was set up in triplicates. Negative control (containing 90 mL de-ionized water and larvae food) as well as positive control (containing 3 mL absolute alcohol, 90 mL de-ionized water and larvae were also set up in triplicates. Both the test

controls were set up and maintained at room temperature (27±2°C). The *Anopheles* larvae in each recovery cup were scooped and transferred by means of small nets into test exposure cups containing the sample solutions and or control, larvae food and de-ionized water. The larvae in the test and controls set up were incubated for a period of 12 and 24 h at room temperature (27±2°C). Therefore, the larvae were gently scooped into small nets, washed with de-ionized water, transferred into recovery cups containing 100 mL of de-ionized water, maintained at pH 7.0 and allowed to settle. Prior to mortality determinations, the larvae in recovery cups were gently disturbed and made to go below the water surface by agitating the water with a sterile pipette. The dead and dying larvae which started to float on the surface, were pushed down the recovery cups. The living larvae which were able to swim to the surface were allowed to do so within 5 min following addition. The larvae remaining and or staying at the bottom of the recovery cups unable to swim to the surface were regarded as dead.

Anti-Microbial Sensitivity Test

Determination of Zone of Inhibition

The media were prepared according to Manufacturers' instructions, poured into sterile petri-dishes (diameter, 13.5 cm) and then allowed to set. The bore-hole diffusion method was used for the anti-microbial screening test. The bacteria were cultured in nutrient agar while the fungus was cultured in the sabouraud dextrose agar. The inoculum of each organism was introduced into each petri-dish. Cylindrical plugs were removed from the agar plates by means of a cork borer to produce wells of approximately 6.0 mm. The wells were equidistant from each other and the edge of the plate (Washington, 1995; National Committee for Clinical Laboratory Standards, 2003). Concentrations of 10 and 20 mg mL^{-1} of the crude ethanolic extract and the fractions at 5 mg mL^{-1} dissolved in methanol: de-ionized water (1:1 v/v) were separately introduced into wells. Also, concentrations of 10 μg mL^{-1} of ampiclox (a standard antibiotic), 1 mg mL^{-1} of ketoconazole (a standard anti-fungal drug) and methanol: de-ionized water (1:1 v/v) were introduced into other wells as positive and negative controls, respectively. The experiments were carried out in triplicates. The plates were left at room temperature (27±2°C) for 2 h to allow for diffusion. The plates were then incubated at 34±2°C for 24 h.

RESULTS AND DISCUSSION

The plant material used in this present study was identified, authenticated and collected observing basic guidelines of plant collection. The solvents and reagents used were of analytical grade. The phytochemical screening revealed the presence of alkaloids, saponins, tannins cardiac glycosides and flavonoids while anthraquinones and terpenes were absent (Table 1). This confirms previous studies as contained in Trease and Evans (1996). Secondary metabolites such as alkaloids, tannins, flavonoids and cardiac glycosides present in the plant are the basis for the curative and or management of many ailments such as wounds, digestive disorders, coughs, ulcers, skin troubles and different kinds of inflammations claimed in its ethno-medicine.

Larvicidal Activity

Preliminary larvicidal assay was carried out on the crude extract at 5 and 10% w/v and at 12 and 24 h incubation. The larvicidal activity (LA%) was calculated in terms of percentage mortality. The lethality furnished after 12 and 24 h incubation was concentration and time-dependent (Table 2, 3). At 5% w/v (12 and 24 h) and 10% w/v (12 and 24 h), the plant demonstrated very weak larvicidal activity of 10 and 20%, 20 and 30%, respectively. These results have revealed that the potential for larvicidal activity is very small in the crude extract of the plant. However, research is presently ongoing in our laboratory to determine if the fractions obtained from the crude extract will

Table 1: Phytochemical screening of leaf extract of *Nymphaea odorata*

Plant metabolite	Test	L
Alkaloids	Dragendoff's	+
	Mayer's	+
Saponins	Froth	+ + +
	Emulsion	+ + +
Tannins	Ferric chloride	+ + +
Cardiac glycosides	Salkowski's	+
	Liebermann's	+
	Burchard's	+
Terpenes	Sulphuric acid	-
Anthraquinones	Borntrager's	-
Flavonoids	Shinoda's	+ + +

L = Leaf extract of *Nymphaea odorata*, + = Trace (insignificant amounts), + + + = Abundant, - = Absent

Table 2: Larvicidal activity (LA%) of leaf extract of *Nymphaea odorata* at 5% w/v after 12 and 24 h incubation

Sample	LA% (12 h)	LA% (24 h)
L	10	20
PC	100	100
NC	0	0

L = Leaf extract of *Nymphaea odorata*, PC = Positive Control, NC = Negative Control

Table 3: Larvicidal activity (LA%) of leaf extract of *Nymphaea odorata* at 10%w/v after 12 and 24 h

Sample	LA% (12 h)	LA% (24 h)
L	20	30
PC	100	100
NC	0	0

L = Leaf extract of *Nymphaea odorata*, PC = Positive Control, NC = Negative Control

Table 4: Anti-microbial sensitivity of leaf extract and fractions of *Nymphaea odorata* at different concentrations in methanol: De-ionized water (1:1 v/v) *Zone of inhibition±0.5 mm

	L (mg mL^{-1})		L (5 mg mL^{-1})					
Test organism	10	20	Lh	Lc	Lb	A	K	C
B. subtilis	6.0	7.0	6.0	6.5	7.0	25	NT	6
S. aureus	6.0	7.0	6.0	6.5	7.0	29	NT	6
E. coli	6.0	6.5	6.0	6.0	6.5	18	NT	6
S. typhi	6.0	6.5	6.0	6.0	6.5	19	NT	6
K. pneumoniae	7.0	7.0	7.0	7.0	7.5	19	NT	6
C. albicans	7.5	7.5	7.5	7.5	7.5	NT	30	6

L = Leaf extract of *Nymphaea odorata*, Lh, Lc, Lb (hexane, chloroform and butanol fractions of leaf extract, respectively), A = Ampiclox (standard antibiotic or anti-bacterial drug), K = Ketoconazole (standard anti-fungal drug), C = Methanol: De-ionized water (1: 1v/v), NT = Not Tested, *Zone of inhibition recorded is diameter of zone and bore-hole cup size [zone diameter (mm) + 6 mm]

demonstrate any improvements in the larvicidal activity. Interestingly, the crude leaf extract tested positive to both alkaloids and saponins which have been shown in separate studies (Bentley *et al*., 1984; Ojewole *et al*., 2000; Oladimeji *et al*., 2006a, b, 2007; Nia *et al*., 2006) to be lethal to the fourth instar larvae of *Anopheles gambiae* which prevent the emergence of adult mosquitoes responsible for the transmission of malaria still scourging huge populations of people around the world.

Anti-Microbial Sensitivity Test

The extract and the fractions (obtained from the chromatographic purification of the extract) were screened for antibacterial and anti-fungal activities using *B. subtilis*, *S. aureus*, *E. coli*, *S. typhi*, *K. pneumoniae* and *C. albicans* to represent a desirable spectrum of microbes. The extract was tested at 10 and 20 mg mL^{-1} while the fractions were screened at 5 mg mL^{-1} because of the possible higher level of purity associated with fractions. The results presented in Table 4 show that the activities

elicited were slightly concentration-dependent. Generally, the extract and the fractions were inactive against all the microbes tested. Alternatively, it could be inferred that the plant did not elicit any remarkable antibacterial and anti-fungal activities.

These results are surprising because the phytochemical screening carried out on the extract revealed the presence of tannins and flavonoids which have been reported in previous studies (Lamikanra *et al*., 1990; Burapadaja and Bunchoo, 1995; Adesina *et al*., 2000) to be anti-microbial. The present study can not support the claimed uses of the plant in ethno-medicine especially for ailments of microbial origin.

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REFERENCES

Adesina, S.K., O. Idowu, A.O. Ogundaini, H. Oladimeji, T.A. Olugbade, G.O. Onawunmi and M. Pais, 2000. Anti-microbial constituents of the leaves of *Acalypha wilkesiana* and *Acalypha hispida*. Phytother. Res., 14: 371-374.

Bentley, M.D., D.E. Leonard and W.F. Stoddard, 1984. Pyrrolizidine alkaloids as larval feeding deterrents for spruce budworm (*Choristonewa fumiferana*). Ann. Entomol. Soc. Am., 7 (4): 393-397.

Burapadaja, S. and A. Bunchoo, 1995. Anti-microbial activity of tannins from *Terminalia citrina*. Planta Med., 61: 365-366.

George, D.P., 2001. Encyclopaedia of Medicinal Plants. Educational and Health Library, Spain, pp: 246-247.

Gibson, L. and J. Khoury, 1986. Storage and survival of bacteria by ultra-freeze. Lett. Applied Microbiol., 3: 127-129.

Harborne, J.B., 1984. Phytochemical Methods. 2nd Edn. Chapman and Hall, London, pp: 279.

Harvey, W.F. and L. John, 1898. Kings American Dispensary-Nymphaea-Water-Lily. Henriette's Herbal Homepage.

Josh, H. and E. Emily, 2002. Saint Mark's Natural Wildlife Refuge-Florida Nature: *Nymphaea odorata*-Fragrant Water-Lily. St. Mark's FL.

Murray, P., E. Baron, M. Pfaller, F. Tenover and R. Yolken, 1995. Manual of Clinical Microbiology. ASM Press, Washington DC.

National Committee for Clinical Laboratory Standards, 2003. Performance Standard for Anti-Microbial Susceptibility Test. 8ht Edn. Approved Standard. Villanova (PA): The Committee, pp: 130.

Nia, R., H.O. Oladimeji, P.M.E. Ubulom and B.A.J. Ekpo, 2006. Larvicidal and anti-microbial potential of *Anthocleista djalonensis* L. (Gentianaceae). Afr. J. Pharm. Res. Dev., 2 (1): 97-101.

Lamikanra, A., A.O. Ogundaini and F.O. Ogungbamila, 1990. Anti-microbial constituents of *Alchornea cordifolia* leaves. Phytother. Res., 4 (5): 198-200.

Odey, R. and D. Kingdersley, 1993. The Complete Medical Herbal Caw Cabbage. Medicinal Herbs-Online Water -Lily Inc., 232, Madison Avenue, New York.

Ojewole, J.A.O., S. Rahim and F.O. Shode, 2000. Mosquito larvicidal properties of aqueous extracts of *Senna didymobotryra*. Nig. J. Nat. Prod. Med., 4: 46-47.

Oladimeji, H.O., R. Nia and E.J. Edoho, 2006a. Larvicidal and anti-microbial activity of *Byrophllum pinnatum* (Lam.) Oken. Afr. J. Pharm. Res. Dev., 2 (2): 102-108.

Oladimeji, H.O., P.M.E. Ubulom, A.C. Igboasoiyi, K. Ndukwe and R. Nia, 2006b. Some biological activities of *Pycnanthus angolensis* (Welw.) Warb. J. Pharm. Bioresour., 3 (1): 1-7.

Oladimeji, H.O., R. Nia, N. Kalu and E.E. Attih, 2007. *In vitro* biological activities of *Carica papaya*. Res. J. Med. Plant, 1 (3): 92-99.

Sievers, A., F.A. Archer, R.H. Moore and B.R. McGovran, 1949. Insecticidal tests for plants from tropical America. J. Ecol. Entomol., 42: 549.

Sofowora, A., 1993. Medicinal Plants and Traditional Medicine in Africa. Spectrum Books, Limited, Nigeria, pp: 142-157.

Trease, A. and W.C. Evans, 1996. Pharmacognosy. 14th Edn. W.B. Saunders Company, Limited, London, pp: 35-37, 219, 224-228.

Washington, J., 1995. Manual of Clinical Microbiology. Lannelte, E., A. Balows, W.J. Haasler and H.J. Shadomy (Eds.). 4th Edn. American Society of Microbiology, pp: 967-970.

WHO, 1970. Guidelines on Management Strategies of Tropical Infections Diseases. WHO Publications, 443: 73.