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Antibacterial activities of aqueous and ethanolic extracts of *Pistia stratiotes* L.

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ABSTRACT: Aqueous and ethanolic extracts of *Pistia* L. (Water lattuce or duckweed) 'kainuwa' (in Hausa) were *in vitro* assayed for antibacterial activity. The extracts impregnated assayed for antibacterial activity against clinical isolates of *Staphylococcus aureus, Salmonella typhi, Escherichia coli, Klebsiella* sp. *Proteus* sp. and *Pseudomonas aeruginosa*. After inocubation *Staph. aureus, S. typhi* and *E. coli* showed sensitivity to the aqueous extract at disc concentration range of 2.0-20mg/ml, 40-100mg/ml respectively. Both the two extracts however, showed no activity against P*roteus* sp., *Klebsiella* sp and *Pseudomonas aeruginosa.* The observed minimum inhibitory concentrations (MICs) of the aqueous extract for *Staph. aureus, S. typhi* and *E. coli* were respectively, 5.0, 60.0 and 100mg. The study demonstrates some agreement with earlier claims on the ethnomedicinal use of the plant in the treatment of ulcers, fevers, bacterial infection of the eye, ear, nose throat as well as the stomach and skin. More work is thus recommended especially on purification, spectrum of activity and the toxicology of the active constitute(s) of the plant.

Key Words: Medicinal plants; Antimicrobial agents; Phytochemistry; Water lettuce; *Pistia stratiotes*.

Introduction

Pistia stratiotes L., water lettuce, tropical duck weed or "Kainuwa" (Hausa) is an aquatic-floating herb belonging to the family *Archie*. It is a macrophyte with up to 12.5cm rosette of light green leaves clearly veined; obovate; lobed at the tip and hairy toward the base (Javier *et al*; 1995). Small white flowers in spike arise from the bottom of the leaves, as do stolons. *Xanthosoma robustum, Clocasia, Aarisorum, Sautromatum* and *Acorus Calmus* are its close relatives (Kato *et al* 1995). It is a common plant occurring in sheltered waters and most dominant weed in especially, organically polluted fresh waters and streams (Lind and Tallantire, 1975).

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In Nigeria the plant also common in ponds and streams as observed in Kano for the purpose of the present study (Anon. 1999). The plant was said to be raised by jevanese on fishponds because certain edible shrimp species prefers to live below the plant. Cut into pieces and mixed with rice, the plant is valuable as feed for ducks. Among the Chinese the plant is used as sandwich given to swine (Dutta, 1995). Game animals such as buffalo was found to relish the plant (Bernard; 1969). The possibility for using *Pistia stratiotes* for water treatment purposes in Egypt was being examined (khedr *et al;* 1998).

Yearoat (1977) reported that Pistia was ethnomedicinally used in the treatment of throat trouble, mouth and stomach disorder. The leaves of the plant was also applied as decoction to treat boils, sores and abscesses by herbalists. It has also been used to reduce fevers and calm nervous disorders since ancient time.

Likewise, Muzatavon (1983) claimed that the plant possesses antagonistic effect on intestinal bacteria and its leaves are locally useful in the eradication of lice. Moreover, a preliminary exploration by the authors of the present study present study yielded that the prominent use of the plant as medicinal herb was widely known by many traditional healers in Kano.

Recently, Adoum *et al* (1997) observed the antimicrobial activity of its extracts on *S. typhi*, *P aeruginosa*, *Klebsiella*, *E coli* and *Candida albicans*. Only *E. coli* was not inhibited at mic10^3mg. Toxicologically however, when the tissues of *Pistia statiotes* are brought in contact with mocous membrane, an exceedingly irritating sensation due to rephids (needle like hair) of calcium Oxalate was noted (Yun, *et al* 1999). The plant was also observed to accumulate an appreciable amount of calcium from aqueous solution in the polluted water (Rai *et al* 1995).

Bernard (1969) reported that the large quantity (530g), of the plant fed to rabbit exerted a toxicological effect evento fatal results in Kano, Adoum *et al* (1993) also showed that *Pistia stratiotes* leaves was toxic to brime shrimp *Artemic salina.*

Accordingly, to substantiate the reported application of *Pistia stratiotes* L. in ethnomedicine as it relates to microbial infections, it was the objective of the present study to screen *in vitro* the antibacterial activity of the ethanolic and aqueous extracts to the plant on some clinical isolates of bacteria. This was with a view to assessing of the results of the recently published works on the plant so as to establish any possibility for further patenting of its active components fate a detailed pharmacognosy and toxicology scheduled along the line.

Materials and Methods

Collection and Identification of the plant Material

Pistia stratiotes rosettes were collected from ponds within Kano Metropolis in northern Nigeria in the Month of May 1999. The specimen's botanical identity was identified at the Botany Unit of the Department of Biological sciences, Bayero University, Kano with the aid of Botanical Keys (Arber, 1972). Reference plant materials was preserved as an album in the herbarium of the Department. The experimental material was air-dried and ground into fine using powder using pestle and mortar (Plate 1).

Extraction procedure:

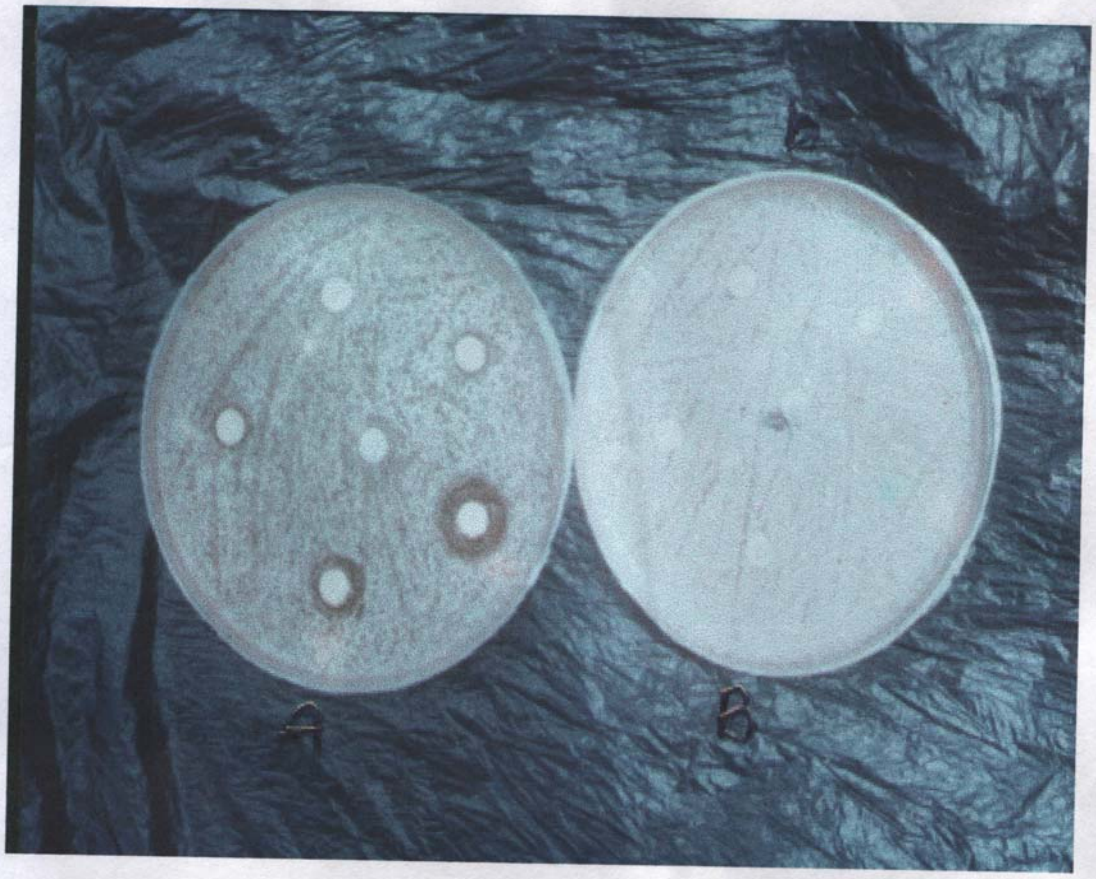
Aqueous Extracts:

Two hundred grams of the powdered sample was mixed with two litres of distilled water in a 2-litre flask and heated at 70^0c for two hours and then allowed to cool 40^0c for the next 46 hours and expressed. The expressed liquid was strained using what man quantitative filter paper No. 1the filtrate was evaporated gently to dryness. The extract was preserved in a clean sample bottle in a deep-freezer (Fatope etal, 1993; Nwafor etal 1996 and Wariso etal 1996).

Ethanol Extract:

The methods of Aishamma and Mistcher (1979) as demonstrated by Mukhtar and Wakili (1999) were followed. The ethanol was achieved by mixing 200g of the dried powdered plant with two litre of ethanol (95% V/V) for 48 hours at room temperature. The mixture was then filtered and the ethanol content in the

Plate 1. Pistia stratiotes L.. (Duck weed) or Water lettuce. Collected from Hauren Shanu Pond near Gadon Kaya, South- Central Kano Metropolis (May 1999)



late 2. Sensitivity of Staphylococcus aureus to aqueous extract of Pistia Stratiotes L. At disc concentrations of 1,2,5,10 and 20mg/ml. (A). As opposed to the respective ethanolic extract shown in B.

filtrate was removed by percolation in vacuo below 40°C. The extract was then evaporated to dryness at 40°C before storage in fresh sample bottles in a refrigerator (0 – 8°C).

Preparation of sensitivity discs:

Impregnated sterile paper discs (Diameter 6.25 ± 0.1 mm) of Whatmann No. 1 filter paper containing the extract at various concentrations, in mg/ml; namely 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 33.3, 40.0, 50.0, 66.6, 80.0 and 100.0 mg/ml for aqueous and ethanol extracts respectively were prepared by employing the methods of Bauer-Kirby (1966) and that of Stokes and Ridgeway (1980) as demonstrated by Deeni and Hussein (1991).

Test Culture

The bacterial pathogens used in the bioassay are *Staphylococcus aureus, Klebsiella* sp., *Salmonella typhi, Proteus* sp., *Escherichia coli* and *Pseudomonas aeruginosa*. These were clinical isolates obtained from Murtala Muhammed Specialist Hospital (MMSH), Kano. The identities of the pathogens were ascertained again in the research laboratory through biochemical and cultural tests according to Mackie and Maccartney (1989).

Culture Media

Mueller Hinton agar and nutrient broth (Oxoid) were used for these investigations.

Preparation of inoculum
An overnight nutrient broth culture of the test organism was used to prepare an inoculum of about 3.3×10^6 cfu/ml. This was arrived at by 1:200 dilution of the culture suspension which matched with the turbidity of 0.5 Macfarlan and barium chloride standard (Deeni and Hussein, 1991). 1:1000 macrobroth dilution was also prepared for the determination of the inhibitory concentration of the test agents.

Bioassay procedure

Agar diffusion and tube methods as outlined by stokes and ridgeway (1980) and as demonstrated by Deeni and Hussein (1991) as well as Wariso et al (1996) were followed. A standard swab of the test inoculum was used to inoculate evenly and aseptically the replicates of Mueller hinton agar plates then allowed to prediffuse for 5minutes with a sterile forceps. Five impregnated sensitivity discs were arranged firmly on to the agar surface. Control discs with no extract were no extracts were also supplied in the control sensitivity plates. These were incubated aerobically at 37^0c $\pm$ 1 for 18 – hrs. Diameters of the zones of inhibition were measured and recorded in millimeters.

Tube or broth dilution method was followed for the determination of the minimum inhibitory concentrations (MICs) of the extracts. The concentration of the extracts used were 0.5, 1.0. 2.5. 5.0, 10, 20, 40,60,80, and 100 mg/ml Deeni and Hussain, (1991). 0.5ml of each of the extract concentration was added to 9.5ml of 1:1000 dilution of inocum in nutrient broth. Control tubes with only 9.5ml of 1:1000 dilution of inocum in nutrient broth and without the extract were also prepared and incubated for 18 – 24 hours at 37^0c $\pm$ 1. Tubes were examined microscopically for turbidity (bacteria growth). Minimum concentration of the extract that inhibited growth (show by no visible turbidity was considered as the MIC.

Results

The observed antibacterial activity of the aqueous and ethanol extracts of *Pistia stratiotes* L. are presented in Table 1 and 2 respectively. The aqueous extract was found to be active against; *Staphylococcus aureus* showing zone of inhibition of 8.5 and 16.0 mm at 2.0 and 20mg/ml disc concentration respectively; *Salmonella typhi* with 6.5 and 7.0 mm zones of inhibition at 40 and 100mg/ml respectively. It was also active against *E. coli* at 80 and 7.0 mm respectively. There was no observable *in vitro* activity against *Proteus, Klebsiella* sp. and *Pseudomonas aeruginosa* (Plate2)

The ethanolic extract on the other hand inhibited *Staph aureus* showing 8.5 and 9mm zone diameter at 50 and 100mg/ml disc concentration were observed for *Salmonella typhi*, it was 6.5mm at 100mg/ml concentration for *E. coli*. There was no activity against *Proteus* sp., *Klebsiella* and *P. aeruginosa*.

The MIC of the aqueous extract was the only one measured as some laboratory constrained prevented the measurement of the MIC of the ethanol extract. The MICs of the aqueous extracts were 5.0, 60.0 and 100mg respectively for *Staph. Aureus, S. typhi* and *E. coli* (Table 3).

Table 1: Antibacterial Activity of Aqueous extract of *Pistia stratiotes* L.

Extract concentration (mg/ml)	ZONE OF INHIBTION (mm)					
	Staph. aureus	*Salmonella typhi*	*E. coli*	*P. aeruginosa*	*Proteus* sp	*Klebsiella* sp
0.0	0.0	00	00	00	00	00
0.5	0.0	00	00	00	00	00
1.0	0.0	00	00	00	00	00
2.0	8.5	00	00	00	00	00
5.0	9.0	00	00	00	00	00
10.0	12.0	00	00	00	00	00
20.0	16.0	00	00	00	00	00
33.3	16.0	00	00	00	00	00
40.0	16.2	6.5	00	00	00	00
50.0	16.0	6.5	00	00	00	00
66.6	-	6.5	00	00	00	00
80	-	7.0	6.5	00	00	00
100	-	7.0	7.0	00	00	00

Table 2: Antibacterial Activity of Ethanolic extract of *Pistia stratiotes* L.

Extract Concentration (mg/ml)	ZONE OF INHIBITION (mm)					
	Staph auerus	*Salmonella typhi*	*E. coli*	*P. aeruginosa*	*Proteus* sp	*Klebsiella* sp
0.0	00	00	00	00	00	00
33.3	0.0	0.0	0.0	0.0	0.0	00
40.0	0.0	0.0	0.0	0.0	0.0	00
50.0	6.5	0.0	0.0	0.0	00	00
66.6	6.5	6.5	0.0	0.0	00	00
80.0	7.0	6.5	0.0	0.0	00	00
100.0	8.0	7.5	6.5	0.0	00	00

Table 3: Minimum inhibitory concentrations (MICs) of the aqueous extract of *Pistia stratiotes* L.

Test organisms	MIC (mg)
Staph. Aureus	5.0
Salmonella typhi	60.0
E. coli	100
P. aqeruginosa	-
Proteus sp.	-
Klebsiella	-

Discussion

The present study demonstrated that aqueous and ethanolic extract of *Pistia stratiotes* posses antibacterial activity on three clinical isolates. However, the extracts appeared more efficacious on *Staphylococcus aureus* and less so to salmonella and *Escherichia coli*, which were least inhibited. The athanolic extract, compared with the aqueous extract, showed activity at much higher concentrations.
The results showed that staphylococcus was also more sensitive compared to *Salmonella typhi* and *E. coli*. Similar report by Adoum *et al* (1991) showed that *E. coli* was completely insensitive at all concentrations.
The greater efficacy exhibited by the aqueous extract relative to the ethanolic extract may imply that, the active agent(s) may be more soluble in water or probably because of the high polar nature of the water might contained different types of bacteriologically active components. This therefore indicates a need for further pharmacological study.

An equally interesting observation made was that, the most susceptible bacteria in the study was *Staphylococcus aureus* which is a Gram positive organism. Probably the extracts are selectively more active on Gram positive, rather than on Gram negative bacteria. Confirmation of this was however outside the scope of this study. The bacteria were found to be susceptible to herbal extracts in most investigations including those of Irobi and Daramola (1994), Mongelli et al (1996), Zheng et al (1996), Warisos et al (1996), Mukhtar and Shua'aibu (1999).

The activity of the extracts against tested organisms can be more vividly visualized if compared to activities of some antimicrobal agent against some standard control stains of bacteria. *Staphylococcus aureus* (ATCC 25923) yield a zone of inhibition of 14-22mm with streptomycin at 10 microgram/disc, 15 – 19mm with vancomycin at 30 microgram disk and 7 – 13mm with polymyxin B. at u/disk (chessbrough, 1984). Zones inhibition produced by staphylococcus aureus in this study are 16 and 8.5mm at disk concentration of 20mg/ml and 2mg/ml respectively (about 200 and 20 microgram/disk as a disc of 6.25mm absorbs approximately 0.01ml of solvent). For *E. coli* (ATCC 25922) zone of inhibition is 12-20mm with streptomycin at 10 micro/disc and 12-16mm with polymyxin B at 300 u/disc (cheesbrough, 1984) E. Coli produced zones of inhibition of 6.5 and 7.5mm at disc concentration of 80 and 100 mg/ml respectively (about 800 and 1000 microgram/disc). *Pseudomonas aeruginosa* (ATCC 27853) is resistant to the antimicrobals mention above and most other antimicrobals. This is consistent with the insensitivity exhibited by *Pseudomonas aeruginosa* in the present study and the very low sensitivity in work of Mukhtar and Shuaibu (1999).

The activity of the extracts against ethanomedicinal use of the plant in treating stomach pain, uclers of the mouth and tongue, eyes diseases and fever as reported by Morton (1961) and some local sources in Kano, Nigeria (Anon, 1999). *Staphylococcus aureus* is implicated in causation of conjunctivities, secondary infection of uclers, antibiotic-associated enteritis and food poisoning (cheesbrough 1984). *Escherichia coli* is an important cause of diarroeal diseases while *Salmonella typhi* is associated with typhoid fever cheesbrough 1984). In this study area the use of the plant in treating rheumatic pains was noted. Thus the extract may have activity against *Streptococcus pyognenes* was not included in this study due to its unavailability at the culture collection centres at the time of this study. The result of the study is not in complete agreement with the report of muzatevone at al (1993) that the plant is an effective antagonist of intestinal bacteria. This is because only 2 enteric bacteria showed some susceptibility to the extracts: however, at much higher concentration (80 – 100mg). Moreover, the most sensitive organism was

not intestinal bacteria Unless the toxicity level of the extracts are established, it may not be easy to recommend the use of much higher concentrations. This was indeed outside the scope of this work.

In spite of this, the result of the present study is of value as we emerged in an era in which research on drugs derived from plants can expected to occupy a prominent position in national priorities. Considering the likely promise the plant *Pistia stratiotes* exhibited, coupled with its relative abundance in the environment, the plant is worth further studying not only for its medicinal value but also for the other promise it holds including nutritive potentials and water purification prospects as observed by khedr et al (1998) in Egypt. This study should among other things pave the way for standardizing the ethanomedicininal utilization of the plant.

It may not be corrected to say that most developing countries including Nigeria are generally reluctant to place any seriousness to official exploration and exploration of herbal medicine despite the fact that the source of starting materials is normally abundant and the chemical and botanical expertise is readily available. It is thus high time for developing countries to join the race for investigating herbal medicines to further miniaturize the gap of industrialisation between them and developed countries among other things. With virtually large unexplored flora in the developing countries they stand the chance of proving many useful drugs from plants that will alleviate human suffering. The result of this work and that of related researchers presents a challenge to any enthusiastic, energetic and highly motivated scientist in Nigeria and other developing countries.

Conclusion

It can at this point be asserted that the aqueous and ethanolic extracts of *Pistia stratiotes* has antibacterial activity which reflects the ethanomedicinal use of the plant and that the activity was high on *Staphylococcus aureus* can be utilized to augment the services of primary health care and to further bring the nation closer to the world of industrialisation. However, further chemical fractionation characterization and spectrum of its antimicrobal effects should be undertaken. An extensive work regarding its toxicity in invertebrates, vertebrates and mammals at *in vitro* and *in vivo* levels should also be encouraged promptly.

Thus the effect of the extracts in fungi, viruses, protozoa, helminthes and viruses should be studied. Moreover, the use of the agents as insecticides should be advocated. The feasibility of integrating its usage into orthodox medicine and the possibility of initiating local pharmaceutical industries that utilises the plant and other indigenous medicinal plants, as raw materials should also be studied. Studies on the nutritive values and water purification potentials are also needed as the plant displayed some characteristic, indicative of such potentials.

References

Adoum, O. A , Dabo, N.T. and Fatope, M.O (1997). Toxicology of *Pistia stratiotes* and its antimicrobial activities. Int. J. Pharmacognosy 35(5): 334 – 337.

aishamma A. and Mistscher, L.E (1979) Comprehensive survey of indigenous plants for potential economic values. Alkaloids and Antimicrobals. J. Nat Pro. 42 (6): 633 – 642.

Arber (1972) Water plant a study of aquatic angiosperms. Weldon Wisely Ltd. Pp 436.

Bauer, A.W., Kirby, W.M.M (1966). Antibiotic susceptivity testing, a standard single disc method. American Journal of Clinical pathology 45; 493.

Bernard, V. and Trum E.C (1969). Common poisonous plant of east Africa first edition pp. 219.

Cheesbrough, M. (1984) Medical laboratory manual for tropical countries, Volume 2. Thetford Press Limited ELBS Cambridgeshire pp. 196 – 205.

Deeni Y.Y and Hussein, H.S.N (1991) Antibiotic Sensitivity testing. Journal of Ethnopharmacognosy. Elsevier Scientific Publishers Ireland Ltd. Pp 91-96.

Dutta A.C (1995) Botany for degree student 5th edition Oxford University press Delhi Bombay madras India p. 670.

Fatope, M.O Ibrahim H. and Takeda Y. 91993) screening of higher plants reputed as pesticide using the brine shrimp Lethality assay. Int J. Pharmacog. 31: 250-254.

Irobi, O.N and Daramola, S.O (1994) Journal of Ethnopharmacol. 42 (1): 39-43.

Javier, Gorga, Pario, C. Jorge Fernando and Wilson P. (1995) Universal Botanical. Journal of Fresh Water Ecology 10(2): 141-149.

Kato, T. Frei, B Heinrich M., (1995) Phytochemistry (Oxford) 41 (4) 1191-1195.

Khedr, Abdel and Hamid (1998). Environmental influence on the distribution of water lettuce in Egypt. Limnologica 28(4) 367 – 363.

Lid L. and Tallantire (Eds.) (1975) Flowering Plants of Uganda. Oxford University Press 2nd. ed. p. 647.

Mackie and Mc Cartney (1989) ed Practical Medical Microbiology. Longman group, U.K 13th ed. pp433.

Mangelli M. Desmarchelien, C. Coussio, J (1995). Revista Argentina de Microbiological 27(4): 199-203.

Morton M. (1981) Ed. History of Botanical Science Academic press London, Toronto Sydney San Francisco pp. 413.

Mukhtar M.D and Wakili S.A (1998) screening for Antimicrobiolocal Activity of Extract of *Garcinia kola*. African Journal of Material and Natural Sciences. Vol. 1:1 p 118-122.

Muzatevon (1983) Antimicrobal activity of pistia extracts. J. Ppharmacognosy 10(2): 140

Nwafor, P.A., Effriam, K.D and Jacks, T.W (1996) aqueous extraction process of back of *Khaya senegalensis*. West Journal of Pharmacognosy and Drug Research 12: 46-50.

Rai, U.N Tripathi R.D Guptam and Chandra P. (1995) Induction of Phylochelatine under cadmium stress in water lettus. J. Phytochemisrty 30 (9) 2007-2026.

StokesJ.E and Ridgeways G.I (1990): Sensitivity testing Techicues Clinical bacteriology. 5th ed. Edward Arnold Publishing.

Wariso, Benjamin A and Wbony O.O (1996) Antimicrobal Activities of crude extract of Nitele *Kalanchoe pinnata* (Lam) pers. West African J. Pharmacology and Drug Res. Cel. 1265-68.

Yearout Fold (1977) Ed. The power of plant. 1st ed. John Murrey Limited London p 135-172.

Yun Ling, Wanseng, H.L. Baozua, yoanglin Z. Junhua (1999). Investigation on the Chemical constituent of Pistia Stratioted L. J Zhangua Zhanyozath 22 (5). 289-290.

Zheng W.F., Tan R.X., L. (1996) Planta Medica 160 – 162.