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(Research Article)

Evaluation of urease inhibitory and free radical scavenging activities of *Microsorum pustulatum* (G. Frost) copel leaves (Polypodiaceae)

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Abstract

Microsorum pustulatum is an epiphytic fern. Young leaves and stems are edible, used in wound healing (ulcer), treatment of inflammation and skin infections. This study is reporting the effect of the leaves extracts and fractions of *Microsorum pustulatum* on the urease enzyme as well as their free radical scavenging activity. The leaves extracts were obtained by successive cold maceration in n-hexane, acetone and 70% aqueous ethanol. Phytochemical screening was done using standard methods. Urease inhibition assay was done using the modified Berthelot method with thiourea as reference standard urease inhibitor. The free radical scavenging activity was assayed using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) with ascorbic acid as reference standard. The organic (AOP) and aqueous (AAP) fractions were obtained by chloroform-water partitioning of the most active acetone extract. The acetone extract (anti-urease=51.6%, DPPH radical scavenging= 49.9%) and its AOP fraction (anti-urease= 43.3%, DPPH radical scavenging= 70.31%) were observed to have good urease inhibition and free radical scavenging effect when compared with standard at the same 1mg/ml test concentration. The trend in median DPPH radical inhibition concentration: AOP (IC50 = 0.09 mg/ml) > AAP (IC50=0.64 mg/ml) was also observed. The phyto-constituent present in the screened leaf extract are saponins, tannins, phlobatannins, phenolic, flavonoids, terpenoids, steroids, carbohydrates. In conclusion the result validates the traditional use of *Microsorum pustulatum* leaves in treatment of disease such as ulcer, chronic inflammation etc. caused by pathogenic microorganism (*Helicobacter pyroli, Proteus mirabilis* etc.) that needs urease for survival and to curb inflammation in oxidative stress state. Also acetone extract of the plant can be used as additive to urea base fertilizer in the development of ecofriendly urease inhibitors and increase in crop production.

Keywords: *Microsorum pustulatum*; Urease inhibitor; Soil nitrogen utilization; Antioxidant

1. Introduction

Urease is a big enzyme that is within amidohydrolase family [1]. Commonly found in bacteria, fungi, algae, plants, some invertebrates and as soil enzyme. They possess nickel-ions as a metalloenzyme of great molecular weight [2]. Urease catalyzes the hydrolysis of urea to form ammonia and carbon dioxide, hence automatically alters the P^H of the surroundings to basicity level as a result of high concentration of ammonia produced. This resultant pH elevation is of diverse clinical and agronomic significance [3]. Although mammalian cell does not produce urease, it enters the mammalian body through different bacteria, especially within the intestine [4]. Urease is a virulence factor for *Helicobacter pyroli, Proteus mirabilis, S. saprophyticus* and *Mycobacterium tuberculosis* among others that are associated with certain diseases in human and animals. Its activity adds up to kidney stones formation, observed in urolithiasis

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that contribute to acute pyelonephritis and other urinary tract infections. It is also implicated in arthritis, gastric intestinal infections and finally the urease disparity that enhances peptic ulcer development [5]. Its soil activity may damage crop yield, in particular when the covering fertilization method is employed. The urea on the surface of the soil transfers ammonia away from rhizosphere during the hydrolysis process because of its volatile nature. This equally impact negatively on the ecosystem, as acid rain or nitrous oxide pollutant [6]. On the other hand, oxidation is a vital process in living organism needed for the production of energy during catabolism. These metabolic processes however usually result in continuous production of free radicals and reactive oxygen (ROS) that are generated by cells during respiration and cell-mediated immune functions [7]. Free radical can also be produced through environmental pollutants, cigarette smoke, automobile exhaust, radiations and pesticides [8]. Due to the lone pair electron in the outer shell of these free radical, they are highly unstable and attack specific biomolecules in the body such as protein, lipid and DNA [9]. This eventually results in lipid peroxidation with clinical pathophysiologic complications in cancer, cardiovascular diseases, aging and inflammatory diseases [8, 10]. Thus free radical scavenging compounds (antioxidants) helps to keep these free radical in check, without being destabilized themselves. They help to promote longevity and good wellbeing. In the area of Urease, it is well known facts that antibiotics are used in treating bacterial infections. However, they have frequently been found to be ineffective [11], due to high resistance. In drug discovery research, medicinal plants still remain the source of novel pharmacological principle for new drug development. The structural multiplicity and differences among the natural products is of great advantage in countering the rate of drug resistance by the pathogenic bacteria. These diversities in compound arrangement makes natural products very treasurable, not only in medical but in agronomic sector as well. These natural products have been a main source of insight in enhancing health, food sustainability for both animal and human life [12,13]. Many of the plants within the family (Polypodiaceae) have been used traditionally for the treatment of wounds, ulcer, urinary tract disorder, viral, bacterial, fungal, antioxidant and in inflammatory states. This present study used an *in vitro* bioactivity guided approach on assessment of the anti-urease and free radical scavenging activities of the leaves of *Microsorum pustulatum* (Polypodiaceae).

Figure 1 Leaves of *Microsorum pustulatum*

2. Material and methods

2.1. Materials

Reagents and solvents used in this study were of analytical grade and are products of JHD & Sigma Alderich Chemicals. Thiourea as reference standard and Urease test kits (Agape diagnostics Switzerland GmbH), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ascorbic acid as reference standard.

2.2. Sample collection and extraction

The leaves of *Microsorum pustulatum* was collected from the palm tree trunk in Rivers State University at Diobu in Obiakpor Local Government Area of Rivers State, Nigeria. The sample was identified by the Taxonomist with voucher specimen (UPH/P/256) deposited in the herbarium in the Department of Pharmacognosy and Phytotherapy University of Port Harcourt. The plant material was air-dried for two weeks at room temperature and pulverized. A 200g quantity of the pulverized sample was cold macerated by successive extraction with n-hexane, acetone and 70% aqueous ethanol. For each extraction solvent, the maceration was done for 96 hours with intermittent agitation. Fresh replacement of solvent after every 48 hours was done. The combined extracts were filtered and the filtrate was concentrated using a rotary evaporator and the yield obtained was noted.

2.3. Phytochemical Screening

Phytochemical screening was done on dried powdered sample and extracts obtained according to [14, 15]. The analysis was carried out to detect phyto-constituents such as alkaloids, tannins, flavonoids, steroids, saponins, triterpenoids, and carbohydrates.

2.4. APPROVAL

University of Port Harcourt Office of Research Management and Development, Research Ethic Committee (UPH/CEREMAD/REC/MM76/033) approved the work.

2.5. Urease inhibitory analysis of the three extracts

The test was based on the modified Berthelot method [16]. Briefly, the three extracts n-hexane, acetone and 70% aqueous ethanol gotten after successive extraction, were used for the assay at the same tested concentration of 1mg/ml. Thiourea a known urease inhibitor was used as reference standard. The enzyme activity was measured using the spectrophotometer by measuring change in absorbance in the presence or absence of an inhibitor at 625nm using the spectrophotometer. The reaction mixture (in the test tubes) containing enzyme (1ml), test sample solution (1ml) and urea(10 µl), was incubated for 10mins at 37^0c, followed by the addition of a color developer (1ml) and further incubated for another 10 minutes, then 1ml of distilled water was added and the absorbance was taken. The percentage of urease inhibition was calculated using the formula:

$$\text{\% Inhibition of Urease} = \frac{Absorbance\ of\ control - Absorbance\ of\ test\ sample}{\text{Absorbance of control}} \text{ x } 100$$

2.6. Free radical scavenging assays

The DPPH radical scavenging assay was employed [17,18]. A 1 ml aliquot of the various extracts solution (1mg/ml) in triplicate were separately added to 1ml of DPPH (0.05g/100ml of ethanol). The mixture was left in the dark for 30 min before reading at 517nm with ethanol as blank. The negative control contains ethanol in place of extract and ascorbic acid as reference standard. Radical scavenging activity was expressed as percentage and calculated using the formula:

$$\text{\% Scavenging} = \frac{\text{Absorbance of negative control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \text{ x } 100$$

2.7. Fractionation of the acetone extract

The most active acetone extract was partitioned into aqueous soluble (polar phase coded APP) and chloroform soluble (organic phase coded AOP) fractions using separating funnel.

2.8. Biological evaluation of the APP and AOP fractions from the bioactive acetone extract

The two fractions APP and AOP obtained were assayed for urease and free radical scavenging-inhibitory activity as stated earlier procedures. Thereafter, for test extracts with promising activity (% inhibition > 50 %), dilutions of the extracts (0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0) mg/ml in triplicate were separately for DPPH radical scavenging activity as outlined above. The median or half maximal inhibition concentration (IC_{50}) was then determined from regression analysis of a plot of % DPPH inhibition against concentration.

3. Results

Table 1 Result of phytochemical screening of the *Microsorum pustulatum* leaves

Screened phytochemical constituents	n-hexane extract	Acetone Extract	70 % aqueous ethanol extract
Alkaloid			
Dragendorff test	-	-	-
Mayer's test	-	-	-
Hager's test	-	-	-
Phenolics			
$FeCl_3$ test	-	+	+
Flavonoid tests	-	+	+
Shinoda			
AlCl3	-	+	+
Phlobatannin test	-	+	+
Anthraquinone(Borntrager's test)			
Free anthraquinones	-	-	-
Combined anthraquinones	-	-	-
Saponins			
Frothing test	-	-	+
Emulsion test	-	-	+
Cyanogenic glycosides	-	-	-
Triterpenoid			
Liebermann-Buchard test	+	+	-
Salkowski test	+	+	-
Cardiac glycosides			
Kedde's test	-	-	-
Keller-Killiani's test	-	-	-
Carbohydrates			
Molisch test	-	+	+
Fehling's test	-	+	+

Key: + means positive - Means negative

3.1. Bioactivity of fractions from acetone extract

Table 2 Result showing Antiurease activity of extracts and fractions (at 1 mg/ml test concentration) from *Microsorum pustulatum* leaves

Sample	% inhibition Mean ±SEM
Thiourea (Reference standard)	66.7 ± 0.370
N-hexane extract 1	24.1 ± 0.071
Acetone extract	51.6* ± 0.30
70% aqueous ethanol extract	17.1 ± 0.071
AOP	43.3 * ± 0.353
APP	12.0 ± 0.136

Key: * represent the values that are significantly difference from the control at p ≤ 0.05. Number of determination = 3. AAP = aqueous phase of acetone extract, AOP= Organic phase of acetone extract

3.2. Bioactivity of *Microsorum pustulatum* using DPPH

Table 3 Result showing free radical scavenging activity of extracts and fractions (at 1 mg/ml test concentration) from *Microsorum pustulatum* leaves

Sample	% inhibition Mean ±SEM
Ascorbic acid (Reference standard)	72.70± 0.268
N-hexane extract	0.65± 0.163
Acetone extract	49.9± 0.319
70% aqueous ethanol extract	12.0 ± 0.169
AOP	70.31± 0.157
APP	62.20± 0.454

Table 4 Concentration-dependent free radical scavenging activity profile of the organic (AOP) and water soluble (AAP) phase from the bioactive acetone extract of *M. pustulatum* leaves

Conc(mg/ml)	% inhibition organic Phase Mean± SEM	% inhibition Aqueous phase Mean ±SEM
0.1	51.97± 0.401	23.49± 0.130
0.2	56.20± 0.230	28.34± 0.394
0.4	60.63± 0.401	40.16± 0.454
0.6	61.81± 0.232	48.95± 0.524
0.8	64.96± 0.261	53.02± 0.130
1.0	70.31± 0.157	62.20± 0.454

± - Standard Error of triplicates. Difference between values are significant at $p< 0.05$ with two way ANOVA test

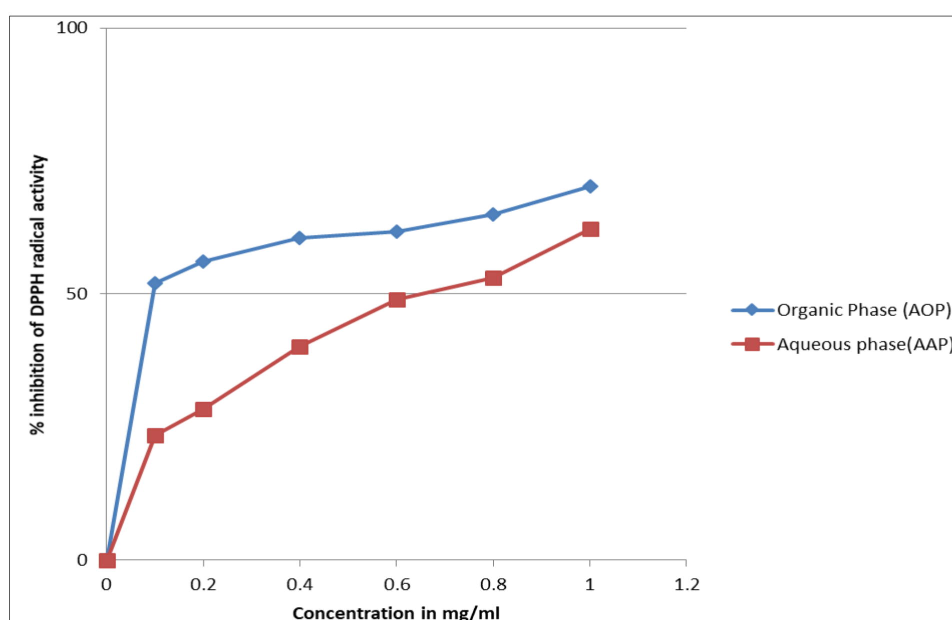

Figure 2 Concentration-response curve for the free radical scavenging activity profile of the organic (AOP) and aqueous soluble (AAP) phase from the bioactive acetone extract of *M. pustulatum* leaves

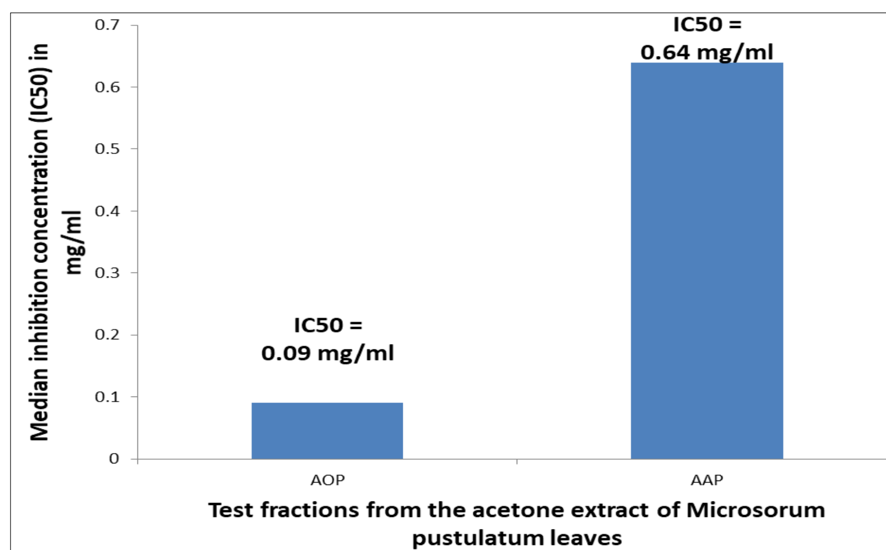

Figure 3 Median inhibition concentration- (IC_{50}) for the free radical scavenging activity profile of the organic (AOP) and aqueous soluble (AAP) phase from the bioactive acetone extract of *M. pustulatum* leaves

4. Discussion

Clinical significance of ureases has become an important therapeutic goal for the treatment of disease caused by a pathogenic bacterium that requires them to survive. This is because the rate of antibiotics ineffectiveness in treating bacterial infection is quiet alarming [11] due to the resistance they develop towards them. Also from the Agricultural perspective, it is of significant interest to discover and develop ecofriendly urease inhibitors as additives for urea base fertilizer. In drug and agrochemicals discovery research, medicinal plants still remain the source of novel pharmacological principle for new drugs and agrochemical development. The study shows bioactivity guided anti-urease assay conducted on the three extracts (n-hexane, acetone and 70% aqueous ethanol). The acetone extract was the most active of the three with a mean % urease inhibition of 51.6 % though significantly ($p < 0.05$) lower compared to the reference thiourea (see Table 2). Being the most active, the acetone extract was further subjected to solvent-solvent partitioning between chloroform (organic) and water (aqueous) to afford the two fraction AOP (organic phase) and APP(aqueous phase). However the trend in its urease inhibition activity appears to reduce with fractionation as its daughter fractions AOP (43.3 %) and AAP (12.0%) were significantly ($p <0.05$) lower. This could probably be due to a synergistic effect among the constituents of the acetone extract. With respect to the antioxidant (DPPH free radical scavenging) activity, the acetone extract was also the most active. However unlike as observed for the urease inhibition activity, its two daughter fraction AOP and AAP were more active that the parent acetone extract with acetone extract (see Table 3). Generally, the AOP was observed to be significantly ($p < 0.05$) more active with respect to both the urease inhibition (see Table 2) and the free radical scavenging (see Tables 3 and 4, Figures 2 and 3) activities at the same test concentrations. The trend in median DPPH radical inhibition concentration: AOP (IC_{50} = 0.09 mg/ml) > AAP (IC_{50}=0.64 mg/ml) was also observed. This is an indication that the bioactive constituent is/are concentrated within the AOP –the organic chloroform soluble phase of the acetone extract. The phytochemical screening result in Table 1 shows the presence of phenolic, flavonoids and carbohydrates in both acetone and 70% aqueous ethanol extracts while saponins are present only in the 70% aqueous extract of the leaves. The presence of triterpenoids was observed only in n- hexane and acetone extracts. The presence of triterpenoid observed in the leaves (n-hexane extract) is in line with species of this genus *Microsorum* reported to contain phytoecdysteriod [19]. Steroids are biosynthetically classified as triterpenoids. Ecdysteriod has many pharmacological influences on Human and other mammals such as anabolic, hypoglycemic, hypocholesterolemic, tonic, hepatoprotective, antidepressant and purgative effects [20, 21]. Phenolic (flavonoids) on the other hand possess antinflamatory, anti-atherosclerotic, antiulcer, antihepatotoxic, anthithrombogenic, antiosteoporotic, anttumor, antiviral, antibacterial, antifungal activity [22]. Presence of saponins in 70% aqueous ethanol possess antioxidant effect on the skin and protect it against UV damage, which attest to its traditional use for skin protection. Phlobatanins and other forms of tannins are capable of forming a complex compound by binding to protein and cellulose [23]. Pharmacologicaly, tannins possess antibacterial, antioxidant and anticancer activities through cellular oxidative damage inhibition [24]. So, the antiurease and antioxidant effect observed can be attributed to the presence of these phyto-constituent in the extract.

5. Conclusion

Microsorum pustulatum leaves in this study have urease inhibitory and free radical scavenging potentials which could be attributed to the presence of the phto-constituents such as phenolics and triterpenoids present. This has confirmed scientifically the use of this fern in ethno medicine for the management of inflammatory states, wound healing (ulcer) and urinary tract infections.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare a no conflict of interest in this work.

References

[1] Sander C, Holm L. An evolutionary treasure: unification of a broad set of amidohydrolases related to urease. Proteins: Structure, Function, and Bioinformatics. 1997; 28(1): 72-82.

[2] Krajewska B, Zaborska W, Leszko M. Inhibition of chitosan immobilized urease by boric acid integrated method. Journal of molecular catalysis B: Enzymatic. 1997; 3: 231-238.

[3] Mobley HLT, Hausinger RP. Microbial ureases: significance, regulation, and molecular characterization. Microbiology Reviews. 1989; 53: 85–108.

[4] Stepankov AA, Kuznetsova TA, Vecherskii MV. Urease activity in gastro-intestinal tract of the European hare (Lepus europaeus). Biology bulletin. 2017; 44(2): 224-227.

[5] Hausinger RP, Mobley HL, Island MD. Molecular biology of microbial ureases. Microbiological reviews. 1995; 59(3): 451-480.

[6] Artola E, Cruchaga S, Ariz I, Moran JF, Garnica M, Houdusse F. Effect of N-(n-butyl) thiophosphoric triamide on urea metabolism and the assimilation of ammonium by Triticum aestivum L. Plant Growth Regulation. 2011; 63: 73–79.

[7] Sharma P, Jha AB, Dubey RS, Pessarakli M. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal of botany. 2012; 217037: 1-26.

[8] Masoko P, Eloff JN. Screening of Twenty-Four South African Combretum and Six Terminalia Species (Combretaceae) for Antioxidant Activitiies. African Journal of Traditional Complementary and Alternative Medicine. 2007; 4(2): 231-239.

[9] Nagmoti DM, Khatri DK, Juvekar PR, Juvekar AR. Antioxidant activity and free radical-scavenging potential of Pithecellobium dulce Benth seed extracts. Free Radical Anttioxidants. 2012; 2l: 37-43.

[10] Hodzic Z, Pasalic H, Memisevic A, Srabovic M, Saletovic M, Poljakovic M. The influence of total phenols content on antioxidant capacity in the whole grain extracts. European Journal of Scientific Research. 2009; 28(3): 471-477.

[11] Choudhary A, Bechtold ML, Puli SR, Othman MO, Roy PK. Role of prophylactic antibiotics in laparoscopic cholecystectomy: a meta-analysis. Journal of Gastrointestinal Surgery. 2008; 12(11): 1847-1853.

[12] Rice MJ, Legg M, Powell KA. Natural production in agriculture- a view from the industry. Pesticide Science. 1998; 52(2): 184-188.

[13] Cameron KC, Di HJ, Moir JL. Nitrogen losses from the soil/plant system: a review. Annals of Applied Biology. 2013; 162: 145–173.

[14] Harborne JB. Phytochemical methods – a guide to modern techniques of plant analysis. 5th ed. London Chapman and Hall. 1998.p. 21-72.

[15] Houghton PJ, Raman A. Laboratory Hand book for the fractionation of Natural Extracts. Chapman and Hall.London,UK, 1998.39.p.12.

[16] Weatherburn MW. Phenol hypochlorite reaction for determination of ammonia. Analytical Chemistry. 1967; 39: 971-974.

[17] Juntachote T, Berghofer E. Antioxidative, properties and stability of ethanolic extracts of Holy Basil and Galangal. Food Chemistry. 2005; 92: 193-202.

[18] Afieroho OE, Ajuzie JI, Afieroho MC. Proximate composition and evaluation of some antioxidant properties of the fresh fruits of Harungana madagascariensis Lam. Ex Poir. (Hypericaceae). Research Journal of Food Science and Nutrition. 2019; 4(6): 97-102.

[19] Ho R, Teai T, Loquet D, Bianchini JP, Girault JP, Lafont R, Raharivelomanana P. Phytoecdystreriods in the genus Microsorum (Polypodiaceae) of French Polynesia. Natural Product Communications. 2007; 2(8),1934578x0700200803.

[20] Camps F. Plant ecdysteroids and their interaction with insects. In Ecological Chemistry and Biochemistry of Plant Terpenoids; Harbone JB, Tomas-Barberan FA, Eds., Clarendon Press, Oxford. 1991.p. 331-376.

[21] Slama K, Lafont R. Insect hormones-ecdysteroids: their presence and actions in vertebrates, European Journal of Entomology. 1995; 92: 355-377.

[22] Mondal S, Rahambn ST. Flavonoid: A vital resource in healthcare and medicine. Pharmacy and Pharmacology International Journal. 2020; 8(2): 91-104.

[23] Haslam E. Natural Polyphenols (Vegetable Tannins) as Drugs: Possible Modes of Action. Journal of Natural Products. 1996; 59(2): 205-215.

[24] Bele AA, Jadhav VM, Kadam VJ. Potential of Tannins: A Review. Asian Journal of Plant Science. 2010; 9(4): 209-214.