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Original Article

Antibacterial activity of methanol extract of *Macaranga denticulata* leaves and *in silico* PASS prediction for its six secondary metabolites

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Abstract
Antibacterial properties of methanolic extract of *Macaranga denticulata* leaves was studied on three Gram positive and Four Gram negative bacteria by disc diffusion method. The extract showed zone of inhibition in highest concentration of 1000 µg/ml against Gram-positive bacteria *Staphylococcus aureus* (Nil), *Bacillus subtilis* (12mm), *Bacillus cereus*(Nil)and Gram-negative bacteria *Salmonella typhi* (15mm), *Salmonella paratyphi* (Nil), *Escherichia coli* (14mm), *Pseudomonas aeruginosa* (14mm). Six secondary metabolites of *Macaranga denticulata* namely 3-acetylaleuritolic acid, oleanolic acid, macarangin, scopoletin, β-sitosterol, stigmasterol were analyzed by the PASS for their different types of biological activities and found activities like hepatoprotectant, antiulcerative, antifungal, diuretic, insulin promoter, antinociceptive, anti-inflammatory, antihypercholesterolemic, anesthetic general, bone diseases treatment and vitamin.

Key Words: *Macaranga denticulata*, Gram-positive, Gram-negative, Zone of inhibition, PASS prediction

INTRODUCTION

As per the World Health Organization (WHO), 80 % of the world's populaces depend on traditional medications. The act of home grown drug is regular in rural regions where western drugs are excessively generous or not accessible [1]. Humans have normally used plants to treat common communicable diseases and some of these traditional medicines are still part of the routine treatment of various malady. It has been reported that 115 articles were published on the antimicrobial activity of medicinal plants in PubMed during the period between 1966–1994, but in the following decade, between 1995 and 2004, 307 were published [2]. It is therefore essential for systematic evaluation of plants used in traditional medicine for various ailments. Hence, there is need to screen medicinal plants for promising biological activity [3]. Drugs derived from unmodified natural products or drugs semi-synthetically obtained from natural sources corresponded to 78 % of the new drugs approved by the FDA between 1983 and 1994 [4]. As of late, different medication resistance in human pathogenic microorganisms has grown because of unpredictable utilization of business antimicrobial medications regularly utilized as a part of the treatment of irresistible sicknesses [5]. Separated from this, the vast majority of the engineered antimicrobial operators have different unfavorable consequences for human wellbeing. Despite what might be expected, the plant-determined antimicrobial specialists are not connected with side impacts and they have an imminent remedial advantage to recuperate numerous irresistible illnesses [6]. This condition required scientists to search for new antimicrobial agents from various sources like medicinal plants which are good sources of novel antimicrobial drugs [7]. For the Same, current global populations are as well turned to plant medicines as their first line therapy for combating diseases and for routine health management [8].

Biologically active substances have therapeutic and supplementary actions, the latter manifesting as side effects. Some of the major biological activities of a compound become evident during the initial preclinical studies; others during clinical trains and the rest come to light during the post marketing phase. These newer activities of the compound provide insight for therapeutic applications.

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Prediction of activity spectra for substances (PASS) is hosted by the V. N. Orechovich Institute of Biomedical Chemistry under the aegis of the Russian Foundation of Basic Research. The web-based application predicts the biological activity spectrum of a compound based on its structure. It works on the principle that the biological activity of a compound equates to its structure. PASS prediction tools are constructed using 20000 principal compounds from MDDR database (produced by Accelrys and Prous Science). The database contains over 180000 biologically relevant compounds and is constantly updated.

M. denticulata Muell. Arg. (Euphorbiaceae) is a small to medium-sized, evergreen tree and is a common pioneer species in moist open areas and secondary forests [9]. In the mountains of Northern Thailand, *M. denticulata* is used as a fallow enriching species by Karen hill tribe farmers [10]. In folk medicine, traditional healers use fresh or dried leaves of some Macaranga species to treat swellings, cuts, sores, boils and bruises [11]. A phytochemical review of literatures indicates the genus Macaranga to be a rich source of the Isoprenylated, geranylated and farnesylated flavonoids and stilbenes. Furthermore, more classes of secondary metabolites like terpenes, tannins, coumarins and other types of compounds are known to be isolated from different species of the genus Macaranga. Flavonoids and stilbenes are regarded as the major constituents and are most likely responsible for most of the activities found in the plants of this genus. It is experimentally validated that *M. denticulata* Possess thrombolytic and Cytotoxicity [12].

The aim of the present study to identify the antibacterial activity of methanol extract of *Macaranga denticulata* and also we have described the biological activity of 3-acetylaleuritolic acid, oleanolic acid, macarangin, scopoletin, β-sitosterol, stigmasterol, which were isolated from *M. denticulata*.[13]

METHOD AND MATERIAL

Plant collection: The leaves of *M. denticulata* were collected from the Chittagong city area in front of Chittagong Medical college hostel gate of Bangladesh in October, 2014 then identified by Dr. Sheikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Chittagong, Bangladesh. Voucher specimens, collection id: CTG 121, for *M. denticulata* kept in the Department of Pharmacy, International Islamic University Chittagong, Chawkbazar, Chittagong-4203, Bangladesh for further reference.

Extracts preparation: The collected plant was washed thoroughly with water and air dried for a week at 35 to 40 °C and pulverized in electric grinder. The obtained powder was successively added to methanol with vigorous shaking at 55 to 60 °C temperature. The extracts were made to dry by using rotary evaporator under reduced pressure. The extract was preserved at 4^0 C for further use.

Microorganisms: Seven bacterial species, gram-positive *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus* gram-negative *Salmonella typhi, Salmonella paratyphi, Escherichia coli, Pseudomonas aeruginosa*. These microbes were obtained from the department of Pharmacy International Islamic University Chittagong.

Preparation of sample discs: The sample discs of about 5 mm in diameter were cut by punching machine (Kangaro 280) from Whatman No. 1 filter paper (Made in China). The discs were taken in a Petri dish and sterilized by autoclave (Daihan Labtech Co., LTD Model: LIB-060M: ISO 9001 certified) dried in oven at 180°C.

Standard antibiotic disc: Kanamycin antibiotic disc (Oxoid, England,) with concentrations of 30μg/disc was used as standard to compare with the sample.

Antibacterial assay: The antibacterial assay was performed by using the disc diffusion method [14-15]. Seven pathogenic bacteria were used as test organisms for antibacterial activity of *M. denticulata* extract. The test organisms were inoculated on 10 ml previously sterilized nutrient agar media, mixed thoroughly and transferred immediately to the sterile Petri dish in an aseptic condition using a sterile loop. Prepared sample and standard solutions were applied to the corresponding Petri dish. The plates were incubated for overnight at 37^0 C. After proper incubation, clear zone of inhibition around the point of application of sample solution were measured which is expressed in millimeter (mm).

***In silico* Prediction of activity spectra for substances (PASS):** The biological activity spectra of the secondary metabolites of *M. denticulata* were obtained using the Prediction of Activity Spectra for Substances (PASS) software. PASS prediction tool is constructed using 20,000 principal compounds from the MDDR database (produced by Accelrys and Prous Science). [16] The chemical structures of the 3-acetylaleuritolic acid, oleanolic acid, macarangin, scopoletin, β-sitosterol and stigmasterol were obtained from Pubchem compound repository

(http://www.ncbi.nlm.nih.gov/pccompound). The structures were drawn using the Chem sketch package 11.0 belonging to the ACD chem. Laboratory. The biological activity spectrum was predicted by PASS.

A biological activity spectrum for a substance is a list of biological activity types for which the probability to be revealed (Pa) and the probability not to be revealed (Pi) are calculated. Pa and Pi values are independent and their values vary from 0 to 1. The result of prediction is valuable at planning of the experiment, but one should take into account some additional factors: Particular interest to some kinds of activity, desirable novelty of a substance, available facilities for experimental testing. Actually, each choice is always the compromise between the desirable novelty of studied substance and risk to obtain the negative result in testing. The more is Pa value, the less is the probability of false positives in the set of compounds selected for biological testing. For example, if one selects for testing only compounds for which a particular activity is predicted with Pa≥0.9, the expected probability to find inactive compounds in the selected set is very low, but about 90% of active compounds are missed. If only compounds with Pa ≥ 0.8 are chosen, the probability to find inactive compounds is also low, but about 80% of active compounds are missed etc. By default, in PASS Pa= Pi value is chosen as a threshold, therefore all compounds with Pa>Pi are suggested to be active. Another criterion for selection is the compounds' novelty. If Pa value is high, sometimes one may find close analogues of known biologically active substances among the tested compounds. For example, if Pa > 0.7 the chance to find the activity in experiment is high, but in some cases the compound may occur to be the close analogue of known pharmaceutical agents. If 0.5 < Pa < 0.7 the chance to find the activity in experiment is less, but the compound is not so similar to known pharmaceutical agents. If Pa < 0.5 the chance to find the activity in experiment is even more less, but if it will be confirmed the compound might occur to be a new chemical entity.

RESULTS

Antibacterial assay: Antibacterial activities of the extract were tested against seven pathogenic bacteria and were compared with the standard antibiotic Kanamycin by measuring the zone of inhibition diameter and expressed in millimeter (mm) showed in table 1.

Table 1: Antibacterial activity of Methanolic extracts of *M. denticulata*

Name of the bacteria	Diameter of zone of inhibition (mm)			
	500µg/disc	800µg/disc	1000µg/disc	Standard (Kanamycin) (30µg/disc)
Gram Positive				
Staphylococcus aureus	0	0	0	30
Bacillus subtilis	8	9	12	27
Bacillus cereus	0	0	0	28
Gram Negative				
Salmonella typhi	10	12	15	33
Salmonella paratyphi	0	0	0	30
Escherichia coli	7	11	14	28
Pseudomonas aeruginosa	10	11	14	27

***In silico* Prediction of activity spectra for substances (PASS):** Six secondary metabolites of *M. denticulata* namely 3-acetylaleuritolic acid, oleanolic acid, macarangin, scopoletin, β-sitosterol, stigmasterol were analyzed by the PASS for their different types of biological activity. The results showed 3-acetylaleuritolic acid (Table 2) could possess biological activities like hepatoprotectant, antiulcerative, antifungal and diuretic. Oleanolic acid (Table 3) exhibited Insulin promoter, antinociceptive and anti-inflammatory. Macarangin (Table 4) and scopoletin (Table 5) showed similar effects like anticarcinogenic, antihelmintic, anti-inflammatory, kinase inhibitor etc. β-sitosterol (Table 6) and stigmasterol (Table 7) both possess the activities like antihypercholesterolemic, anesthetic general, antinociceptive, bone diseases treatment and vitamin.

Table 2: PASS results of 3-acetylaleuritolic acid ($C_{32}H_{50}O_4$)

Pa	Pi	Activity
0.939	0.004	Mucomembranous protector
0.928	0.001	Transcription factor stimulant
0.914	0.002	Chemopreventive
0.873	0.003	Hepatoprotectant
0.872	0.003	Oxidoreductase inhibitor
0.874	0.005	Antineoplastic
0.869	0.003	Insulin promoter
0.869	0.005	Apoptosis agonist
0.852	0.005	Hypolipemic
0.851	0.004	Lipid metabolism regulator
0.729	0.005	Antiulcerative
0.718	0.004	Gastrin inhibitor
0.674	0.005	Hepatic disorders treatment
0.662	0.008	Antiviral (Influenza)
0.561	0.022	Antifungal
0.507	0.012	Antitoxic
0.438	0.009	Diuretic
0.454	0.059	Antipruritic, allergic
0.387	0.084	Antiarthritic
0.264	0.019	Thrombolytic

Table 3: PASS results of Oleanolic acid ($C_{30}H_{48}O_3$)

Pa	Pi	Activity
0.987	0.001	Insulin promoter
0.984	0.002	Caspase 3 stimulant
0.961	0.001	Hepatoprotectant
0.954	0.001	Transcription factor stimulant
0.901	0.004	Apoptosis agonist
0.895	0.001	Antinociceptive
0.877	0.005	Antineoplastic
0.836	0.002	Antiviral (Influenza)
0.833	0.006	Hypolipemic
0.831	0.005	Antiinflammatory
0.827	0.003	Antiulcerative
0.809	0.003	Lipid peroxidase inhibitor

0.77	0.002	Protein-tyrosine phosphatase inhibitor
0.709	0.004	Cytoprotectant
0.693	0.01	Vasodilator, peripheral
0.679	0.002	Contraceptive female
0.639	0.004	Diuretic
0.628	0.007	Antileukemic
0.585	0.006	Antimetastatic
0.588	0.021	Cholesterol antagonist
0.575	0.021	Antifungal
0.569	0.019	Antithrombotic
0.517	0.02	Vasodilator

Table 4: PASS results of Macarangin ($C_{25}H_{26}O_6$)

Pa	Pi	Activity
0.987	0.001	UGT1A9 substrate
0.963	0.002	Lipid peroxidase inhibitor
0.961	0.001	Hemostatic
0.958	0.001	UGT1A1 substrate
0.953	0.003	Membrane integrity agonist
0.945	0.001	Free radical scavenger
0.919	0.003	Reductant
0.905	0.002	Chemopreventive
0.907	0.004	Chlordecone reductase inhibitor
0.894	0.004	Apoptosis agonist
0.873	0.004	Kinase inhibitor
0.87	0.003	Anticarcinogenic
0.863	0.003	Antioxidant
0.861	0.003	Antimutagenic
0.835	0.003	Antiulcerative
0.795	0.004	Cardioprotectant
0.773	0.004	Histamine release inhibitor
0.757	0.01	Antiinflammatory
0.752	0.005	Histidine kinase inhibitor
0.705	0.001	Melanin inhibitor
0.684	0.006	Antiparasitic
0.688	0.01	Antifungal
0.672	0.003	Anti-Helicobacter pylori
0.672	0.006	CYP2C8 inhibitor
0.641	0.004	Antihelmintic
0.644	0.014	Antisecretoric
0.629	0.006	Platelet adhesion inhibitor
0.619	0.013	Antithrombotic
0.543	0.013	Antibacterial

Table 5: PASS results of Scopoletin ($C_{10}H_8O_4$)

Pa	Pi	Activity
0.958	0.003	CYP2C12 substrate
0.938	0.003	Chlordecone reductase inhibitor
0.898	0.002	Antimutagenic
0.900	0.004	Aldehyde oxidase inhibitor
0.900	0.011	Membrane integrity agonist
0.890	0.003	Cardiovascular analeptic
0.824	0.004	Spasmolytic, urinary
0.816	0.008	Membrane permeability inhibitor
0.811	0.004	Peroxidase inhibitor
0.774	0.004	General pump inhibitor
0.749	0.005	Antiseptic
0.747	0.008	Vasoprotector
0.750	0.011	Apoptosis agonist
0.746	0.009	Kinase inhibitor
0.692	0.004	Neurotransmitter antagonist
0.702	0.022	Fibrinolytic
0.688	0.015	Respiratory analeptic
0.657	0.009	Hepatoprotectant
0.659	0.012	Radioprotector
0.654	0.018	Membrane integrity antagonist
0.627	0.012	Vasodilator, coronary
0.639	0.024	Antiinflammatory
0.626	0.013	Histidine kinase inhibitor
0.635	0.027	Kidney function stimulant
0.605	0.013	Antihypercholesterolemic
0.585	0.015	Antiprotozoal (Leishmania)
0.575	0.008	Antipyretic
0.570	0.014	Anticarcinogenic
0.560	0.022	Beta glucuronidase inhibitor
0.539	0.003	Melanin inhibitor
0.533	0.012	Antihelmintic (Nematodes)

Table 6: PASS results of β-sitosterol ($C_{29}H_{50}O$)

Pa	Pi	Activity
0.977	0.001	Antihypercholesterolemic
0.965	0.001	DELTA14-sterol reductase inhibitor
0.959	0.002	Prostaglandin-E2 9-reductase inhibitor
0.957	0.001	Cholesterol antagonist
0.933	0.003	Hypolipemic
0.881	0.004	Anesthetic general
0.856	0.004	Dextranase inhibitor
0.849	0.006	Respiratory analeptic

0.717	0.005	Bone diseases treatment
0.708	0.006	Prostate disorders treatment
0.703	0.013	Lipoprotein lipase inhibitor
0.686	0.006	Antiviral (Influenza)
0.674	0.004	Antipruritic, allergic
0.677	0.012	Analeptic
0.667	0.002	Threonine ammonia-lyase inhibitor
0.661	0.000	Secretase alpha stimulant
0.608	0.005	Calcium regulator
0.601	0.006	Hepatic disorders treatment
0.596	0.002	Vitamin
0.588	0.002	Protein synthesis stimulant
0.558	0.014	Antinociceptive
0.547	0.013	Antiviral (Rhinovirus)
0.572	0.038	Antiinflammatory

Table 7: PASS results of Stigmasterol ($C_{29}H_{48}O$)

Pa	Pi	Activity
0.982	0.001	Antihypercholesterolemic
0.965	0.001	Cholesterol antagonist
0.949	0.003	Hypolipemic
0.933	0.001	Oxidoreductase inhibitor
0.827	0.003	Chemopreventive
0.809	0.004	Dermatologic
0.79	0.004	Proliferative diseases treatment
0.788	0.005	UGT1A substrate
0.782	0.007	Immunosuppressant
0.775	0.004	Adenomatous polyposis treatment
0.755	0.004	Antitoxic
0.75	0.004	Antipsoriatic
0.704	0.005	Bone diseases treatment
0.695	0.007	Anesthetic general
0.666	0.017	Respiratory analeptic
0.632	0.013	Dextranase inhibitor
0.614	0.001	Vitamin
0.621	0.009	Antipruritic, allergic
0.613	0.005	Muscular dystrophy treatment
0.617	0.011	Hepatoprotectant
0.601	0.008	Antinociceptive
0.568	0.018	Radioprotector
0.541	0.045	Antiinflammatory
0.489	0.032	Antifungal

DISCUSSION

Plants create a tremendous mixture of optional mixes as characteristic security against microbial and creepy crawly assault. Some of these mixes are dangerous to creatures; be that as it may others may not be lethal. Undoubtedly, a significant number of these mixes have been utilized as a part of the type of entire plants on the other hand plant concentrates for nourishment or medicinal applications in human[17, 18] because plants are the natural reservoir of many antimicrobial, anticancer agents, analgesics, anti-diarrheal, antifungal as well as various therapeutic activities [19]. Acknowledgement of prescriptions from such plant source as an option type of medicinal services is expanding on the grounds that they are serving as encouraging wellsprings of novel anti-microbial models [20-21]. Some of the phytochemical compounds e.g. glycoside, saponin, tannin, flavonoids, terpenoid, alkaloids, have variously been reported to have antimicrobial activity [22-23].The aim of the study was to evaluate the antibacterial activities of crude methanol extracts of *M. denticulata.* Antibacterial activity of *M. denticulata* leaf methanol extract was studied against three Gram positive and four Gram negative bacteria by disc diffusion method and compared with the standard antibiotic disc of Kanamycin (30µg/disc). All three concentrations not produced zone of inhibition and thus showed different degree of antibacterial activity. It was observed that gram negative bacteria showed greater zone of inhibition than gram negative bacteria to the plant extract. A dose dependent antibacterial activity was also found. With the increase in extract concentration, the zone of inhibition was also increased. However, the highest zone of inhibition was observed in 1000 mg/disc extract for all the strains. For 1000 mg/disc, zone of inhibition was the highest (15 mm) in *Salmonella typhi* and the lowest (12 mm) in *Bacillus subtilis.* For 800 mg/disc, zone of inhibition was highest (12 mm) in *Salmonella typhi* and the lowest (9 mm) in *Bacillus subtilis*. For 500 mg/disc, zone of inhibition was the highest (10 mm) in *Salmonella typhi* and *Pseudomonas aeruginosa* and the lowest (7 mm) in *Escherichia coli*. An inhibition zone of 10mm or greater was considered to indicate good antibacterial activities. The methanol extract was not active against *S. aureus, B. cereus and S. paratyphi.*

In order to accelerate the research for potent natural products, computer-aided drug discovery program PASS was used to predict the biological activity. PASS prediction tools were constructed using 20000 principal compounds [24] and about 4000 kinds of biological activity on the basis of structural formula with mean accuracy about 90%. [25] The result of prediction is presented as the list of activities with appropriate Pa and Pi ratio.

The predicted results for secondary metabolites of *M. denticulata* show the available information on the pharmacological activity/mechanism/effects and were corroborative with previous reports. [12, 26, 27]

Conclusion

This study delineates that *M. denticulata* extract possesses moderate antibacterial effect. Since, crude methanol extract of *M. denticulata* showed antibacterial effect on some bacteria. PASS prediction also compatible with the antibacterial activity of *M. denticulata*. It predicted that secondary metabolite of *M. denticulata* cloud show more antifungal activity rather than antibacterial activity. PASS also predicted many other biological activities like antinociceptive, anti-inflammatory, anticarcinogenic, antihypercholesterolemic etc. So, further studies are necessary to prove these activities and elucidate the mechanism lying with these effects. However, this is the first report on this sample and it may serve as a footstep regarding the biological and pharmacological activities of this sample.

Competing interests: The authors declare that they have no competing interests.

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