



Antimicrobial Activity of Aqueous extracts of *Peperomia pellucida*, *Plectranthus amboinicus* and *Annona muricata*

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Received: 14-Jan-2023, **Manuscript No.** JOCPR-23-87155; **Editor assigned:** 16-Jan-2023, **PreQC No.** JOCPR-23-87155 (PQ); **Reviewed:** 30-Jan-2023, **QC No.** JOCPR-23-87155; **Revised:** 26-Jun-2023, **Manuscript No.** JOCPR-23-87155 (R); **Published:** 03-Jul-2023

ABSTRACT

Since the age of the ground breaking discovery of penicillin, antibiotic resistance has been increasingly more vulnerable, encouraging novel antibiotics. Carpeted with its magnificent antibiotic sources, our mother earth can tackle even more complicated puzzles. The aim of the work is to unveil the antimicrobial properties of *Peperomia pellucida*, *Plectranthus amboinicus* and *Annona muricata*. Fresh extracts were squeezed out using mortar and pestle and the antimicrobial studies were performed against nine strains *Candida albicans*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Shigella flexneri*, *Enterobacter species*, *Serratia marcescens*, *Enterococcus species* and *Staphylococcus aureus* using Kirby-Bauer method at dilutions using water. Most of the samples recorded their optimum concentration as 20%. *Plectranthus amboinicus* displays antibacterial activity (15 mm) equivalent to the control against *Shigella species*. *Annona muricata* exhibits excellent tolerance against *E. coli*, equivalent to the control (16 mm). Antifungal activity of *P. amboinicus* is superior compared to the other plant species in this study. Comparing overall, the aqueous extract of *Plectranthus amboinicus* is comparably more active against the others. Significant activities against all the samples support authentication of novel drugs from the plant species.

Keywords: *Peperomia pellucida*, Antibacterial, *Plectranthus amboinicus*, *Annona muricata*, Antifungal, Leaf extract

INTRODUCTION

With the serendipitous event, antibiotics have been remaining as pillars of healthy humanity. But non-judicial use of these magnetic bullets contributes to the growing problem of antibiotic resistance. Recently, it has been increasingly gaining attention as the number of multi-drug resistant species is emerging nowadays. Posing a major threat to global health, research is being sped up every year in search of an effective alternate.

Peperomia pellucida, a Piperaceae plant, possesses potential pharmacological activity because of the presence of bioactive compounds which include alkaloids, flavonoids, saponin, tannins and cardiac glycosides (Figure 1). Some of the main terpenoid constituents of its essential oil comprise linalool, limonene, β -caryophyllene and linalyl acetate. The methanolic extract possesses angiotensin converting enzyme inhibitory activity and some bioactive compounds secolignan (peperomins), lignin, pellucidin A, patuloside A, dillapiole, sesamin, isoswertisin, chromenes

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and quercetin [1-4].

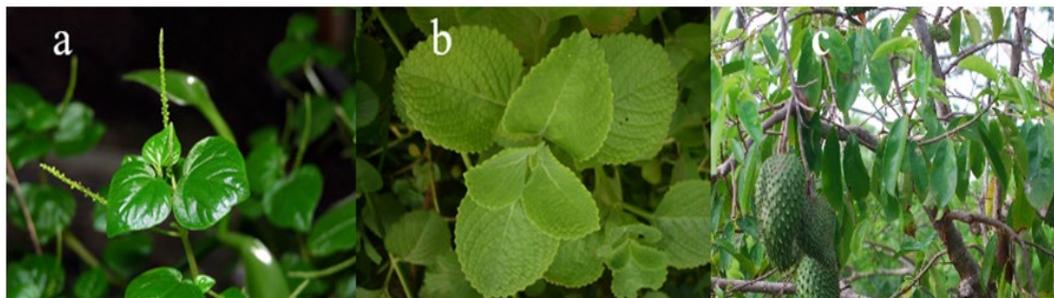


Figure 1 a) *Peperomia pellucida*; b) *Plectranthus amboinicus*; c) *Annona muricata*.

Though the whole plant is being traditionally used for multiple medicinal applications worldwide, some of its bioactivities include anti-tumour, anti-inflammation, hypertension, anti-cancer, antisickling, antiamoebic, bactericide, antioxidant, analgesic, anti-HIV and antifeedant properties. Apart from preparing delicious dishes, the succulent fleshy aerial part has been traditionally used to treat fever, cold, viral diseases, asthma, rheumatic pain, kidney infections, gout, hypertension, diabetes mellitus, abdominal pain, venereal diseases and against poisonous insect bites [5-8].

Plectranthus amboinicus, a succulent fleshy plant, belongs to Lamiaceae family. Enriched with its complex bioactive compounds originated from its secondary metabolism comprising 76 volatiles and 30 non-volatile compounds belonging to different classes of phytochemicals (monoterpenoids, diterpenoids, triterpenoids, sesquiterpenoids, phenolics, flavonoids, alcohols, aldehydes and esters), it exhibits many pharmacological activities such as antimicrobial, antitumor, cytotoxic, DNA protective, antidiabetic, antiinflammatory, wound healing, antioxidant, anti-epileptic, larvicidal and analgesic activities [9-14].

The spicy plant being the source of Linalool, p-cymene, β -Cymene, β -Ocimene, 1,8-cineol, gamma-terpinene, 4-terpineol, Carvacrol, Alpha-cisbergamoteno, β -Myrcene, α -Caryophyllene, Caryophyllene oxide Nerol acetate, Geranyl acetate, Nerol is effective against fever, stiff neck, backache, nervous, respiratory, digestive, cardiovascular, oral, skin, and urinary diseases [15,16].

Annona muricata belongs to Annonaceae family enriched with defense chemical compounds acetogenin, annonacin, annomuricin, muricatocin, β -caryophyllene, β -pinene, germacrene D, ρ -mentha-2,4(8)-diene, α -pinene, δ -cadinene, epi- α -cadinol, α -cadinol and β -elemene from the leaf oil [17-20]. It also possesses some isoquinoline alkaloids reticuline, coclaurine, coreximine, atherosperminine, stepharine, anomurine and anomuricine in the leaf, stem and root of the plant.

The Annonaceae species packed with its secondary metabolites saponins, alkaloids and triterpenoids, sesquiterpenes, flavonoids, anthraquinones, tannins, and cardiac glycosides, has been renowned for its anticancerous property since ancient days. Apart from this, it possesses properties anti-hyperglycemic, anti-hyperlipidemic, anticonvulsant, anti-arthritic, antioxidant, antitumour, cytotoxic, antiparasitic, antimalarial, antibacterial, insecticidal, molluscicidal, antiviral, hepatoprotective, hypotensive, antidepressant, wound healing, immunomodulatory and antidiabetic activities.

The potential activities of the plants are yet to be explored more; the present study focuses on their antimicrobial activity against some selected microbial strains.

MATERIALS AND METHODS

Collection and preparation of plant extracts

The fresh young leaves of the plant samples were collected and grounded well using mortar and pestle. The fresh juicy extracts were squeezed out, filtered using Whatmann no.1 filter paper, refrigerated and used for antimicrobial activity.

Tested microorganisms

The crude aqueous extracts of *Peperomia pellucida*, *Plectranthus amboinicus* and *Annona muricata* were tested for their antimicrobial activity against eight different bacterial strains *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Shigella flexneri*, *Enterobacter* species, *Serratia marcescens*, *Enterococcus* species and *Staphylococcus aureus* as well as against a fungal species *Candida albicans* at 20%, 30% and 50% of its aqueous dilutions (v/v) using agar disk diffusion method (Kirby-Bauer method). For antibacterial studies, the control used was Amikacin and for antifungal studies, Nystatin was the control.

RESULTS AND DISCUSSION

The aqueous dilutions of all the three plant species exhibit significant antibacterial and antifungal activity against all the nine microbial strains *C. albicans*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Shigella flexneri*, *Enterobacter* species, *Serratia marcescens*, *Enterococcus* species and *Staphylococcus aureus* (Figure 2).

Peperomia pellucida shows a decrease in activity with an increase in its concentration mostly against both gram positive and gram negative bacterial strains recording a maximum activity at 20% (Figure 3). Similarly, *Plectranthus amboinicus* shows a slight decrease in activity against the gram positive *Enterobacter* and *S. flexneri* strains with its maximum activity at 20%. Again, other than *S. aureus* and *K. pneumoniae* strains, in most cases 20% concentration is enough to impart its maximum activity. A similar trend was observed in *A. muricata* extract against *E. coli* and *S. marcescens*. A higher concentration is required for the extract against *Enterobacter*, *S. flexneri*, *C. albicans* and *S. aureus*.

The report reveals a dependence of antimicrobial activity on its concentration of bioactive compounds. Plants packed full of bioactive secondary metabolites, purely every compound will show a difference in its activity. Here, the decrease in activity after a particular concentration may be because of the dominance of some toxic compounds because of the synergic effect. Also, for any compound after a particular dosage, the activity gets reversed and its toxicological effect dominates. As per this study, the optimum concentration of *P. pellucida* against the gram positive *S. aureus* is 20% and against *Enterococcus* species is 30%. As for *P. amboinicus*, no such negative effects were observed against those bacterial strains. Against *S. aureus*, *A. muricata* shows no negative effects even at 50% but the optimum dosage against *Enterococcus* is 30%.

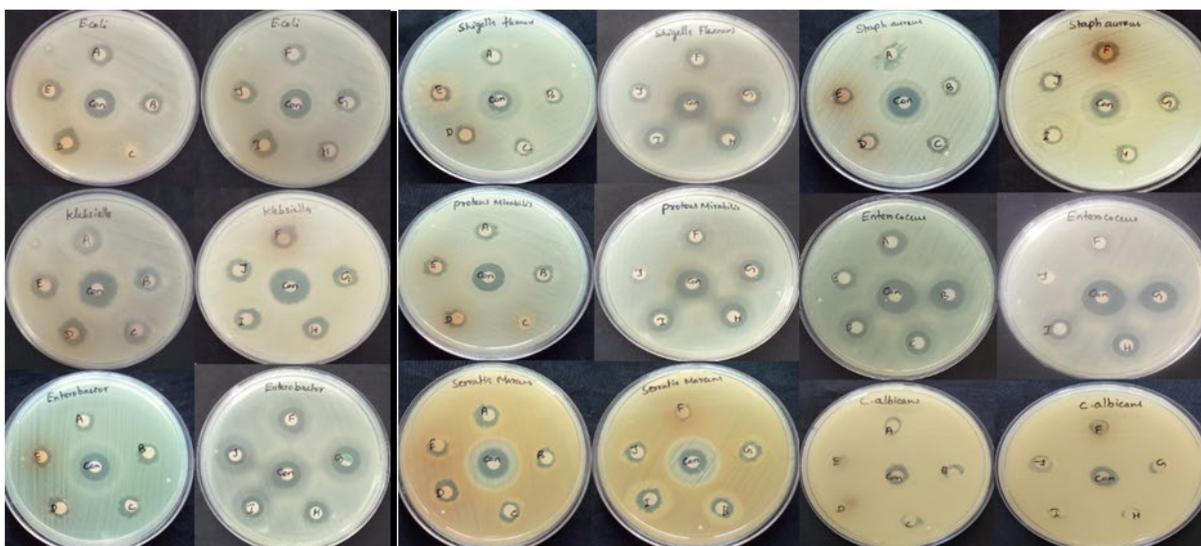


Figure 2 Antimicrobial activity of *Peperomia pellucida* (A-20%, B-30%, C-50%), *Annona muricata* (D-20%, E-30%, F-50%), *Plectranthus amboinicus* (G-20%, H-30%, I-50%).

Against *Enterobacteriaceae* pathogens, *P. pellucida* proves to have its optimum concentration of 20%. *P. amboinicus* is having an optimum concentration of 20% against some species *Enterobacter*, *Shigella* and *Serratia* whereas *A. muricata* is not having any negative effects even at its 50% concentration against the two species *Enterobacter* and *Shigella*. Against other pathogens, here also 20% concentration remains as its optimum. Against fungal species also, *P. amboinicus* is not imparting any negative effects even at its 50% concentration. The finding assures the safe usage of the plant species against various ailments.

Belonging to opportunistic pathogenic variety thriving in hospital environments, *S. aureus* mainly infects immunocompromised patients through an unhygienic mode of food processing, transport and intake or from the products of livestock animals. Being a facultative intracellular pathogen, *S. aureus* has a stunning ability to gain resistance against beta lactam class antibiotics apart from vancomycin. Against this multidrug resistance species, *P. amboinicus* exhibits its optimum activity (17 mm) comparable with the control (20 mm). Also, *A. muricata* exhibits appreciable activity (16 mm) at a higher concentration, whereas *P. pellucida* imparts a comparable activity even at its minimum concentration (Figure 3). Against *Enterococcus*, which possesses tolerance against vancomycin, both the plant species *P. pellucida* and *P. amboinicus* impart comparable activity (17 mm for *P. pellucida* and 16 mm for *P. amboinicus*) relatively at a lower concentration (Table 1).

Against all the gram negative bacterial species, *P. pellucida* shows comparable susceptibility even at its minimum concentration (20%). Against *E. coli*, *S. flexneri* and *P. mirabilis*, higher activity is recorded by *P. amboinicus* at its lower concentration (30%). A remarkable finding is that against *E. coli*, *A. muricata* extract imparts an equivalent activity (16 mm) as that of the control even at its minimum concentration. Again, *P. amboinicus* exerts equivalent activity (15 mm) as that of the control at its lowest concentration against *S. flexneri*; also, the plant shows an excellent activity (15 mm) against *E. coli* almost comparable to the control (16 mm). Susceptibility against *P. mirabilis* is also recorded to be comparable (15 mm) to the plant species. *A. muricata* shows appreciable activity (15 mm) against *K. pneumoniae* and *Enterobacter* species.

Antimicrobial resistance is mainly attributed to its ability to degrade the particular antibiotic with its enzymatic releases. In addition, its capability to change its receptor or its membrane permeability contributes to its antibiotic tolerance. Antibiotics that emerged during the last two decades mostly belong to modified versions of beta lactam class which rendered the bacterial species, especially *Enterococcus*, *S. aureus*, *K. pneumoniae* and *E. coli* to develop quicker resistance. The tested extracts show a comparable activity (17 mm) against both *Enterococcus* and *S. aureus*.

Almost equivalent susceptibility is recorded against the species *E. coli* (15 mm against 16 mm for control). Again, a good antibacterial activity is observed against *K. pneumoniae* species also (16 mm for *P. pellucida* at 20%).

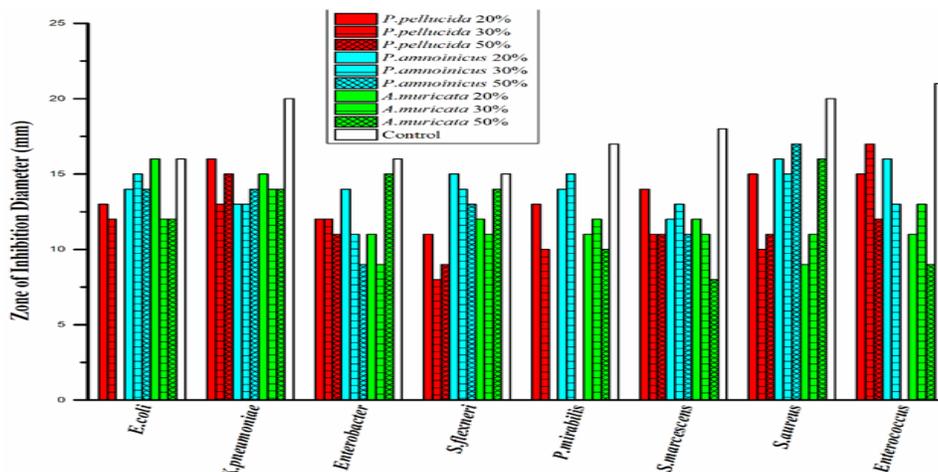


Figure 3: Antibacterial activity of *Peperomia pellucida*, *Plectranthus amboinicus* and *Annona muricata*.

Table 1: Antimicrobial activity of *Peperomia pellucida*, *Plectranthus amboinicus* and *Annona muricata*.

Zone of inhibition diameter (mm)										
Microbial strains	<i>Peperomia pellucida</i>			<i>Plectranthus amboinicus</i>			<i>Annona muricata</i>			Control (Amikacin /Nystatin)
	20%	30%	50%	20%	30%	50%	20%	30%	50%	
<i>E. coli</i>	13	12	-	14	15	14	16	12	12	16
<i>Klebsiella pneumoniae</i>	16	13	15	13	13	14	15	14	14	20
<i>Enterobacter</i>	12	12	11	14	11	09	11	09	15	16
<i>Shigella flexneri</i>	11	08	09	15	14	13	12	11	14	15
<i>Proteus mirabilis</i>	13	10	-	14	15	-	11	12	10	17
<i>Serratia marcescens</i>	14	11	11	12	13	11	12	11	08	18
<i>Staphylococcus aureus</i>	15	10	11	16	15	17	09	11	16	20
<i>Enterococcus</i>	15	17	12	16	13	-	11	13	09	21
<i>Candida albicans</i>	11	11	10	12	13	15	-	-	11	22

Antibacterial activity of *P. amboinicus* is because of its rich secondary metabolites monoterpenes such as thymol, carvacrol and linanol though its chemical composition differs based on its geographical distribution. In India, the chemical composition of some of the main components in the essential oils present in leaves has been reported as carvacrol (43.1%), thymol (7.2%), eugenol (6.4%), chavicol (5.3%) and Et-salicylate (3.2%). In addition, p-Cymene (6.5%-12.6%), β -Caryophyllene (7.4%), caryophyllene oxide (2.2%) and α -Terpinene (5.9%-15.5%) also reported in other studies. Apart from this, all these compounds contribute excellently for antifungal activity also which can account for its good susceptibility (15 mm) against *C. albicans* species.

P. pellucida a glossy plant, has been reported to have bioactive peperomins A, B, C and E apart from lignans, alkaloids, tannins, resins, steroids, flavonoids, phytosterols, steroids, terpenoids, triterpenoids, glycosides and phenolic compounds. These bioactive compounds contribute to its broad spectrum antimicrobial activity at its minimum concentration especially.

Composing of around two hundred and twelve bioactive compounds, the predominant contribution for the antimicrobial activity of *A. muricata* is because of its acetogenins followed by alkaloids like reticuline, coreximine, etc. sesquiterpenes and other phenolic compounds.

Enterobacteriaceae produces broad spectrum β -lactamase enzyme which renders it active against most of the penicillin and cephalosporin class drugs. A recent study by the centers for disease control and prevention published in the year 2019, estimates cases resistant to carbapenem drug also. The present study reveals an encouraging report which estimates a fair amount of activity against all the *Enterobacteriaceae* species.

Another consideration being natural drugs are easier to be processed and metabolized apart from their cost of production, all three plant species being very common in occurrence can be considered for further research which can explore more pharmacologically bioactive compounds.

CONCLUSION

All the selected plant species exhibit a broad spectrum antimicrobial activity because of their diverse and rich bioactive compounds. With the urgency of novel antibiotic emergence, the present study focusing on the antimicrobial activities of the plant species *Peperomia pellucida*, *Plectranthus amboinicus* and *Annona muricata* reveals novel drugs can authenticate from all the plant species. *P. amboinicus* possessing an excellent broad spectrum antimicrobial activity, *P. pellucida* act better against *K. pneumoniae*, *S. marcescens*, *S. aureus* and *Enterococcus* and *A. muricata* show its rich potentiality against *E. coli*, *K. pneumoniae*, *Enterobacter* and *S. aureus*. The phytochemistry of *P. pellucida* is still lagging, which requires careful scrutinizing and analysis.

ACKNOWLEDGEMENT

Non-applicable

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