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Antimicrobial activity of Mystroxylon aethiopicum and Psidium guajava leaves on selected pathogenic bacteria

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***Abstract*---**With the rise of antimicrobial resistance, the urge of having new lead compounds that could inhibit microbial growth is turning into a truth. The aim of the present investigation was to determine the antimicrobial activity of Mystroxylon aethiopicum and Psidium guajava on selected pathogenic bacteria. Extraction with petroleum ether and methanol was performed on both leaf powders. Agar well diffusion technique was followed. All the experiments were carried out in duplicate, and the results were analyzed by SPSS. Glycosides, tannins, saponins, flavonoids, and phenols were seen in both plants. However, alkaloids were only observed in P. guajava. Extracts showed various activities against tested bacteria. P. guajava methanol leaf extracts exhibited the greatest inhibitory activity against all the tested bacteria. The methanol and petroleum ether leaf extracts of M. aethiopicum had no effect on S. pneumonia and E. coli. P. guajava methanol extract had the lowest minimum inhibitory concentration of 50 mg/ml against S. aureus and K. oxytoca. 75 mg/ml was recorded for M. aethiopicum methanol leaf extract against K. oxytoca and S. aureus. The combination of the two extracts also showed a reduced of antimicrobial effect. As M. aethiopicum and P. guajava leaves revealed

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to possess active compounds against pathogenic bacteria, they can be exploited to cure some diseases like urinary tract, wound and nosocomial infections caused by these bacteria. All the plant parts analysis and in vivo and toxicity investigations have to be conducted in order to see the possibility of manufacturing a cost-effective drug.

Keywords---antimicrobial activity, phytocompounds, Psidium guajava, Mystroxylon aethiopicum, inhibition zone, pathogenic bacteria.

Introduction

Most of the pathogenic microbial species have developed resistance towards available commercial synthetic antibacterial and antifungal agents (Frieri *et al.,* 2017). Medicinal plants have been documented to possess different and various secondary metabolites that could act as vital antimicrobial agents for the present and the next generation (Gorlenko *et al.,* 2020). They thus contain bioactive compounds acting as curing agents (Pandith *et al.,* 2018). Some of these compounds are tannins, flavonoids, alkaloids, and terpenoids (Kilonzo *et al.,* 2017). A tropic *P. guajava* and an ornamental garden *M. aethiopicum*, are the trees possessing antimicrobial properties (Kilonzo *et al.,* 2016). *M. aethiopicum* is generally found in African countries (Gwali *et al.,* 2010), while *P. guajava* is found in every corner of the globe and is native in Latino America (Nair *et al.,* 2007). For instance, Celastraceae *M. aethiopicum* extracts can cure the sneezes, urinary tract and respiratory infections, gonorrhea, diarrhea, hypertension and stomach pain due to the presence of saponins, tannins, flavonoids and alkaloids (Cheruiyot *et al.,* 2019). *P. guajava*, a Myrtaceae family, possess various active components acting as controlling agents of various diseases, like burns, vomiting, sore throats, diarrhea, coughs, malaria, tooth pain, and gastroenteritis (Nair *et al.,* 2007). This could be attributed to the presence of high amounts of flavonoids, tannins, cineol and triterpenes in the *P. guajava* essential oil (Azizan *et al.,* 2020).

Pathogenic bacteria have been implicated in most of human diseases and the most alarming aspect has been the rate of increased drug resistance (Reuben *et al.,* 2013). Although most bacteria are harmless and beneficial, some others like *Klebsiella oxytoca*, *Escherichia coli*, *Streptococcus pneumonia*, and *Staphylococcus aureus* are pathogenic (Appelbaum, 2012). Urinary tract and wound infections are frequently caused by *K. oxytoca*. *S. pneumoniae* leads to sepsis in infants, ear and sinus infections, pneumonia in HIV patients, and meningitis (Karimi et *al.,* 2019). Water-borne diseases usually resulted from *E. coli*' action and effect. *S. aureus* is known to cause hospital infections, bacteremia, infective endocarditis, pneumonia, osteomyelitis, food poisoning, etc. (Turner *et al.,* 2019). Various polar and non-polar solvents are utilized to extract phytochemicals from different plant parts with antimicrobial and antifungal activities. For instance, water, ethanol and methanol extracts of *P. guajava* had antibacterial activity against *Salmonella anatum*, *Vibrio parahaemolyticus*, *E. coli* and *Bacillus subtilis* (Shaheena *et al.*, 2019). The stem barks of *M. aethiopicum* are used by Tanzanian ethnic groups for the treatment of different disorders that are infectious including diarrhea (Boer *et al.,* 2005).

Worldwide population are utilizing herbal medicines for centuries owing to its safety and cost-effectiveness (Kilonzo *et al.,* 2017). Microbial resistance to currently commercial active antibiotics has emerged scientists to search for alternatives from medicinal plants with antibacterial and antifungal properties (Gorlenko *et al.,* 2020). Medicinal plant has been documented to possess a rich library of secondary metabolites that could be the potential source of the next generation of antibiotics but not fully explored. Therefore, this research was conducted to investigate the antibacterial effects of *M. aethiopicum* and *P. guajava* phytocompounds on four pathogenic bacteria causing various diseases to Rwandan population.

Materials and Procedures

Plants, bacteria and chemicals

M. aethiopicum and *P. guajava* used in the present investigation were collected from INES-Ruhengeri botanic garden, located in Musanze district of Rwanda. Four bacterial strains utilized were obtained from microbiology department at Rwanda biomedical center. They were *E. coli* ATTCC 35218, *S. aureus* ATTCC 29213, *S. pneumonia* ATTCC 49619, and *K. oxytoca* ATTCC 700524. Culture media and chemicals were bought from HiMedia lab (Mumbai, India) and Sigma-Aldrich (St Louis, USA). Some of them were Mueller-Hinton agar, Mayor`s reagent, nutrient agar, ferric chloride, brain heart infusion broth, petroleum ether, and methanol. All the chemicals were of analytical grade.

Preparation of the plant extracts

The collected two plants were cleaned and rinsed separately using tap water and distilled water, respectively, and then air dried. An electric grinder was used to grind the dried leaves into a fine powder. Chemicals were extracted using the maceration method, with a sample/solvent ratio of 1:10 (w/v). Indeed, 20 g of the powder was taken in 200 ml of 95% methanol for 72 h to obtain the extract. The extraction was performed at room temperature (25 °C) with frequent agitation at 200 rpm (Orbital shaker / OS-340C). The methanol leaf extract was filtered, condensed, concentrated with the help of a rotary evaporator (Heidolph rotary/Laborota 4010 digital) at 40-75 °C, and then stored at – 4 °C. The concentration of the extract was assumed to be 100 mg/ml (Umuhoza *et al.*, 2021; Habyarimana *et al.*, 2022). The procedure was repeated with 95% petroleum ether in the same conditions.

Qualitative analysis of phytosubstances

Chuku *et al.* (2020) method was followed to check the existence of tannins, flavonoids, alkaloids, saponins, phenols, and glycosides. The phytochemicals from both petroleum ether and methanol leaf extracts were individually analyzed with two negative controls, viz. sample and reagent.

Preparation of the inoculum

The inoculums were prepared from *E. coli* ATTCC 35218, *S. aureus* ATTCC 29213, *S. pneumonia* ATTCC 49619, and *K. oxytoca* ATTCC 700524 stored in the Brain heart infusion (BHI) agar slants at 4 °C. The pathogenic bacterial isolates were separately sub cultured with nutrient broth and incubated at 37 °C overnight. Normal saline, with 0.5 Mc Farland standard, was utilized to get 10^8 cfu/ml used in the subsequent experiments.

Antibacterial susceptibility testing of *M. aethiopicum* and *P. guajava*

Antimicrobial susceptibility investigation of *M. aethiopicum* and *P. guajava* extracts was performed following the agar well-diffusion process. Different Mueller Hinton plates were spread with different text pathogenic bacteria. A sterile borer was utilized to punch 0.6 mm diameter wells into the plates. The holes were differently inoculated with *M. aethiopicum* and *P. guajava* methanol extracts (50 *µl* of 100 mg/ml per well). The inoculated plates were left to stand for one hour for proper diffusion. Pure solvents and discs of antibiotics (ciproflaxin and levoflaxin, 5 µg) were used as controls. The Mueller Hinton plates were then incubated at 37 °C. The inhibition zones were measured in mm after overnight incubation. For the evaluation of minimum inhibitory concentration (MIC), 25, 50, and 75 mg/ml plant extracts were utilized. The incubation was carried out in the same conditions. The combined effect of *M. aethiopicum* and *P. guajava* leaf extracts were also studied by considering 1:1 ratio and carrying the experiments in the same conditions. The inhibition zones were then compared when the plant extracts were used singly or in a mixture.

Statistical analysis

The experiments were conducted in duplicate. To compare the means of different extract parameters, the data were subjected to two-way analysis of variance (ANOVA) and the t-test, as well as the Post-Hoc test to assess the differences between variables, where appropriate. A statistically significant P-value was less than 0.05. All these analyses were automatically given by SPSS version 23.

Results and Discussion

Crude extracts of *M. aethiopicum* and *P. guajava* leaves

Extraction is the initial stage in the study of medicinal plants since it is required to isolate and characterize the necessary chemical components from the plant materials (Azizan *et al.,* 2020). High yield extracts were obtained from methanol solvents, compared to the petroleum ether solvent. Both alcoholic extracts exhibited dark green colours. The leaves used had to be dried first in order to decrease the water content of fresh leaves. This method is important to stabilize the bioactive components in their exact form. The type of solvent used in the extraction process determines the effectiveness of extracting biologically active compounds from the plant material (Shaikh *et al.*, 2022). The choice of solvent used is very important as it will allow the isolation of bioactive compound of interest (Kafle *et al.,* 2018).

Phytochemical analysis of *M. aethiopicum* and *P. guajava* leaves

Medicinal plants possess essential sources of producing valuable secondary metabolite compounds which are vital to the well-being of persons as they could be potential source of the next generation of antibiotics (Farhana *et al.,* 2017; Kilonzo *et al.,* 2017). Flavonoids, saponins, alkaloids, tannins, phenols, and glycosides were found in *M. aethiopicum* during a qualitative study for phytochemicals (Table 1).. Similar results were reported by Kilonzo *et al.* (2016) and Gakunga *et al.* (2014) who confirmed the existence of flavonoids, saponins, tannins, phenols, alkaloids and glycosides in *M. aethiopicum.* Likewise, *P. guajava* extracts revealed the presence of pharmacologically active ingredients. These are flavonoids, tannins, saponins, phenols and glycosides. However, the alkaloids were absent (Table 1). Similarly, tannins, saponins, phenols, flavonoids and glycosides were found in the leaf extracts of *P. guajava* (Yagoub *et al.,* 2007; Biswas *et al.,* 2013; Azizan *et al.,* 2020). In contrast, previous studies reported the absence of tannins in *P. guajava* extracts (Sanche *et al.,* 2005; Nair *et al.,* 2007; Kafle *et al.,* 2018). This difference in metabolites could be attributed to geographic, ecological, and genetic differences like plant's origin, age, part used, genetic material, etc.

Table 1
Phytochemicals screened from extracts of *M. aethiopicum* and *P. guajava* leaves

Phytocompound	*M. aethiopicum*	*P. guajava*
Saponins	+	+
Tannins	+	+
Alkaloids	+	-
Flavonoids	+	+
Glycoside	+	+
Phenols	+	+

Antimicrobial activity of *M. aethiopicum* and *P. guajava* leaf extracts

The plant metabolites potentially manage various conditions causing harm to the humankind because they possess various modes of actions (Farhana *et al.,* 2017). The antimicrobial resistance of many currently available antimicrobial agents is increasing, necessitating the development of new drugs with inexpensive raw materials like plant extracts (Kilonzo *et al.,* 2017). In the present investigation, the antibacterial effect of *E. coli* ATTCC 35218, *S. aureus* ATTCC 29213, *S. pneumonia* ATTCC 49619, and *K. oxytoca* ATTCC 700524 was assessed. The results in the table 2 show that the *P. guajava* extracts have a significant antibacterial activity, as shown by their inhibition zones. It was noticed that methanol leaf extract has a stronger inhibitory effect than petroleum ether extract. Methanol extract exhibited the strongest antimicrobial activity against *S. aureus* with diameter inhibition zone (DIZ) of 20 mm followed by *K. oxytoca* (18.5 mm), *E. coli* (17.5 mm) and *S. pneumonia* (16.5 mm) (Table 2). Similarly, a study conducted by Sahel *et al.* (2020) showed inhibition zones of 18 and 21 mm against *E. coli* and *S. aureus*, respectively by using *P. guajava* extract. Likewise, at a concentration of 100 mg/ml, *P. guajava* extract proved effective against *E. coli* (Nair et al., 2007). *P. guajava* extract at a concentration of 100 mg/ml was active against *E. coli* (Nair *et*

al., 2007). Furthermore, Azizan *et al.* (2020) reported that the leaves of *P. guajava* were highly effective against *S. aureus*, *E. coli*, *B. cereus*, and *S. enteritidis* when petroleum ether was utilized as extracting solvent. However, no effect seen against *E. coli* and *S. pneumonia*. With methanol, Umuhoza et al. (2021) extracted *Kalanchoe integra* phytochemicals that were effective in inhibiting all the 4 studied bacteria.

Table 2
Antibacterial activity of *P. guajava* and *M. aethiopicum* on selected pathogenic bacteria

Microorganism	Zone of inhibition (mm)			
	P. guajava		*M. aethiopicum*	
	Methanol	Petroleum ether	Methanol	Petroleum ether
E. coli 35218	17.5 ± 1.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
K. oxytoca 700524	18.5 ± 1.0	16.0 ± 1.0	18.0 ± 1.3	14.0 ± 0.8
S. aureus 43300	20.0 ± 1.4	12.0 ± 0.5	12.0 ± 0.7	10.0 ± 0.3
S. pneumonia 49619	16.5 ± 0.9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Thus, the extracts from *P. guajava* contained pharmacology active compounds due to the presence of saponins, phenols, tannins, glycosides and flavonoids which could be responsible for antimicrobial activity (Sanche *et al.,* 2005; Nair *et al.,* 2007; Eghare *et al.,* 2010; Biswas *et al.,* 2013; Kafle *et al.,* 2018; Azizan *et al.,* 2020; Saleh *et al.,* 2020). These groups of compounds have been shown to be curative against a variety of pathogens, which may explain their widespread use in conventional medicine for a variety of ailments. The inhibition of pathogenic bacterial species can be ascribed to the polyphenolic tannins that bind to proline-rich proteins and interfere with bacterial protein synthesis. The hydroxylated polyphenolic flavonoids also kill pathogenic bacteria to form complexes with soluble or cell wall bacterial proteins (Sahel *et al.*, 2020). Besides, the difference in the antimicrobial activities between organic extracts of the two studied plants can be attributed to several factors such as environmental, seasonal, geographic factors, extraction or laboratory techniques.

S. aureus and *K. oxytoca* were inhibited by *M. aethiopicum* methanol and petroleum ether extracts, with inhibition zone ranges of 12-18 and 10-14 mm, respectively. However, *E. coli* ATCC 35218 and *S. pneumonia* ATCC 49619 were resistant to both extracts (Table 2). Similarly, the leaves extract of *M. aethiopicum* were not sensitive to *E. coli* and *S. pneumonia* (Kilonzo *et al.*, 2016). This difference in antibacterial resistivity and action could be due to differences in cell wall constituents. The antibacterial activity of *M. aethiopicum* leaf extracts could be ascribed to the existence of flavonoids, phenols, and tannin present in them as reported by Gakunga *et al.* (2014). *P. guajava* extracts were compared to *M. aethiopicum* extracts using two-way analysis of variance, which demonstrated a statistically significant difference with a P-value of 0.052937. Similarly, with a P-value of 0.21517, *M. aethiopicum* extracts showed no statistically significant difference when compared to *P. guajava* (Tables 3 and 4). The plant leaf extracts of *P. guajava* and *M. aethiopicum* can be thus be used to treat urinary tract

infections caused by *K. oxytoca*, or nosocomial infection and pus-causing wounds that may result from *S. aureus*. The results presented in the table 3 indicate that the extracts from *P. guajava* exhibited statistically significant different where the P<0.05, while in the table 4, P > 0.05 was regarded as non-statistically significant.

Table 3
Antibacterial activity of *P. guajava* leaves extracts analysis by Two way ANOVA. SS = sum of squared, DF = degree of freedom, MS = mean square, F = ratio of two mean square, F- crit = F critical

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Solvents	247.5313	1	247.5313	9.663684	0.052937	10.12796
Bacteria	133.8438	3	44.61458	1.741765	0.329892	9.276628
Error	76.84375	3	25.61458			
Total	458.2188	7				

Table 4
Antimicrobial activity of *M. aethiopicum* leaves extracts from two different solvents

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Solvents	4.5	1	4.5	2.45455	0.21517	10.128
Bacteria	389.5	3	129.833	70.8182	0.00278	9.27663
Error	5.5	3	1.83333			
Total	399.5	7				

Screening for the minimum inhibitory concentration

MIC values are used to determine which class of chemicals (such as antibiotics) is the most successful, as well as to assess the effectiveness of new antibacterial medicines. (Gorlenko *et al.,* 2020). Extracts that showed high antibacterial activities were diluted to investigate their extent of activity. 50 mg/ml was recorded as MIC for *P. guajava* extracts. It showed antimicrobial activity against *S. aureus* ATCC43300 and *K. oxytoca* ATCC49619. However, 75 mg/ml was MIC for leaf extract obtained from *M. aethiopicum*. This concentration was enough to inhibit both *S. aureus* ATCC43300 and *K. oxytoca* ATCC49619. Similar MIC against *S. aureus* and *K. oxytoca* were reported for the methanol leaf extracts from *M. aethiopicum* (Kilonzo *et al.,* 2016) and from *P. guajava* (Kafle *et al.*, 2018). MIC value noticed for *S. pneumonia* was contradictory (Kafle *et al.*, 2018).

Synergistic activity of *P. guajava* and *M. aethiopicum* extracts on selected pathogenic bacteria

Different plant parts or plant species may be combined to achieve a high level of effectiveness or synergistic or additive effects in acting as antimicrobial agents (Rachuonyo *et al.,* 2019). Table 5 shows the combination of *M. aethiopicum* and *P. guajava* methanol leaf extracts. With t-test, there was no significant different, for both combined plant extracts and plant extracts used singly, in the sensitivity of

tested strain bacteria to the different extracts between methanol and petroleum ether solvent (P > 0.05) (table 6). When the plant extracts were combined, they had less antibacterial activity against *E. coli*, *K. oxytoca*, *S. aureus*, and *S. pneumonia* than when they were used separately. No previous reports available on the antimicrobial activity against all tested bacteria toward the combined effect of *P. guajava* and *M. aethiopicum* extracts. In contrast, an important antibacterial activity was seen when the phyotosubstances of ginger and Turmeric were combined (Rachuonyo *et al.,* 2019). Thus, the combination of secondary products present in plants may enhance or diminish the antimicrobial activity effectiveness.

Table 5
Antibacterial activity of two combined (synergetic) plant extracts on selected pathogenic bacteria

Microorganisms	Methanol	Petroleum ether
E. coli 35218	13.0 ± 1.0	10.0 ± 0.7
K. oxytoca 49619	16.0 ± 0.8	13.8 ± 0.2
S. aureus 43300	18.5.0 ± 1.2	12.7 ± 1.0
S. pneumonia 49619	14.5 ± 0.9	9.5 ± 0.3

Table 6
T-Test for combined plant extracts and plant extract used singly among the solvents

Solvents	P-value	T-cal	T-tab	Observation
PMM vs PM	0.117	1.88	2.5705	not significant
PME vs PE	0.362	1.058	3.182	not significant
PMM vs MM	0.183	1.72	3.182	not significant
PME vs ME	0.212	1.483	2.77	not significant

Key: PMM- *Psidium*+ *Mystroxylon* methanol extract, PM- *Psidium* methanol extract, PME- *Psidium*+ *Mystroxylon* petroleum ether, MM- *Mystroxylon* methanol extract, PE- *Psidium* petroleum ether, ME- *Mystroxylon* petroleum ether.

Conclusion

The antibacterial activity of *P. guajava* and *M. aethiopicum* extracts has confirmed the medicinal value of the 2 plants. They can be used to treat various infections caused by the studied pathogenic bacteria. Further studies have to be conducted to quantify the level of phytochemicals and to evaluate their effectiveness in vivo. Cost-effectiveness has to be checked with other solvents, all plant parts and extraction techniques. Toxicity studies have to be carried out before commercialization.

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