Phytochemical profile, antioxidant and antibacterial activities of *Psidium guajava, Cassia occidentalis, Euphorbia hirta*, and *Todalia asiatica* growing in Comoros islands

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Abstract: Phytochemical content and antioxidant and antimicrobial activities of four plants, *Psidium guajava (P. guajava), Cassia occidentalis (C. occidentalis), Euphorbia hirta (E. hirta),* and *Todalia asiatica (T. asiatica),* used in traditional medinine in the islands of Comoros have been undertaken. The result of phytochemical screening showed the presence of flavonoids and tannins in all extracts except the ethyl acetate extracts of *C. occidentalis* and *T. asiatica*, and the absence of alkaloids in all extracts. The antioxidant capacities of ethyl acetate, and methanol extracts were examined by three different methods, namely, free radical scavenging activity DPPH method, reducing power scavenging activity, and total antioxidant capacity. The tests showed significant dose-dependent antioxidant activity. For DPPH radical scavenging activity and reducing power assay, it is the *E. hirta* extract, which manifested the highest activity. However, for the total antioxidant capacity, the stronger activity was showed by *P. guajava* extract and ethyl acetate extract of *T. asiatica*. *In vitro* antimicrobial studies of methanol extracts were carried out against four medically important microbial strains, including *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas* using disc diffusion assay. The extracts showed a low effect on bacterial growth inhibition.

Keywords: Antibacterial activity, Antioxidant activity, Comoros; Flavonoids content, Polyphenols content.

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Introduction

All living organisms produce their energy by oxidation processes. These processes are controlled by several cellular mechanisms, which produced diverse molecules named reactive oxygen species, including free radicals and their precursors (Halliwell and Gutteridge 2015). The excessive production of free radicals and the unbalanced mechanisms of antioxidant protection resulting in oxidative stress are partly responsible for a great number of age-related diseases such as cancer, cardiovascular disorders, and neurodegenerative diseases (Favier 2003; Sarikurkcu et al. 2009). Among the most interesting scientific research today is the discovery of phytochemical substances, which have protective effects against cell oxidation (Rawson et al. 2014). Antioxidants have been studied and proven to decrease the likelihood of developing these diseases (Dizdaroglu et al. 2002; Kuhn 2003; Temple 2000). The primary metabolic processes, which generally follow a common mechanism in the cells of all plants and are required for each plant to live and reproduce, are considered the fundamental biochemical processes of plants (Kumar et al. 2014). In addition, plants, including herbs, spices and trees, possess a large number of compounds called secondary metabolites that have important pharmacological activities such as anticancer, antibacterial and antioxidant (Asaolu et al. 2009; Nayak et al. 2020; Andrade et al 2018).

Folk medicine in Comoros Islands is rich and diversified, based on natural products due to its mixing African Bantu and Arab-Muslim (Soidrou et al. 2013). It was handed down from generation to generation orally (Kaou et al. 2008). Even though medicinal plants take an important place in this health system, they are not sufficiently documented (Soidrou et al. 2013). Many diseases in the title islands are still treated with conventional medicine, including medicinal plants. Plants such as *Cassia occidentalis*, *Euphorbia hirta*, and *Psidium guajava* are used to a large degree to treat diseases such as malaria, fever, headaches, gynecological and dermal diseases (Soulé et al. 2014).

In the continuity of our previous studies (Soulé et al. 2014; Soulé et al. 2017), we aim to investigate the chemical constituents, total phenolic and flavonoids contents, the antioxidant properties, and antibacterial activities of *Psidium guajava*, *Cassia occidentalis*, *Euphorbia hirta*, and *Todalia asiatica*.

Material and methods

1. Plant material

Cassia occidentalis was harvested in Bouni Hamahamet village (N/E of Ngazidja Island), *Euphorbia hirta*, *Psidium guajava,* and *Todalia asiatica* were harvested in Itsandra region particularly in Dimadjou for *Euphorbia hirta*, Bahani for *Psidium guajava* and *Todalia asiatica* in January 2018. Voucher specimens identified by Ms. Madame Ramadhoini Ali Islam has been deposited in the Herbarium of Department of Botany of Faculty of Sciences and Technology, University of Comoros. The voucher specimens' number is 2025, 1016, 3056 and 3360, respectively for *Cassia occidentalis*, *Euphorbia hirta, Psidium guajava,* and *Todalia asiatica*.

2. Preparation of plant extracts

Dried and ground leaves (without their flowered tops) of *Cassia occidentalis, Psidium guajava, Todalia asiatica and* aerial part of *Euphorbia hirta* were placed in Soxhlet extractor. Briefly, three solvents were successively used according to their polarity (hexane, ethyl acetate, and methanol) and the extracts were concentrated by evaporation on rotavapor. The extracts were stored at 4°C for different tests.

3. Phytochemical screening

A chemical screening of the four plants was first performed in order to know the occurrence of some families of chemical constituents. This phytochemical screening was performed on crushed leaves according to standard procedures. Flavonoids, tannins, and alkaloids were determined according to the works of El Hajaji *et al.* and Soidrou *et al.* using cyanidin reactant for flavonoids, ferrous chloride for tannins, and Dragendorff's, Mayer's and Wagner's reagents for alkaloids (Trease and Evans, 1989; El Hajaji et al. 2011; Soidrou et al. 2013).

4. Total phenolic and flavonoids contents

4.1. Total phenolic determination

Total polyphenols were determined according to the method we early described (El Hajaji et al. 2011). This consists in a colorimetric method using the Folin-Ciocalteu reagent based on oxidation/reduction of phenolic compounds. Briefly, in alkaline medium, polyphenols reduce

the phosphomolybdic acid of Folin-Ciocalteu, this reduction leads to the appearance of a blue color measured at 765 nm. Gallic acid was used as standard.

4.2. Total flavonoids content determination

Total flavonoids content was determined using a spectrophotometric method as described in Soidrou's study (Soidrou et al. 2013). This method is based on the complexation of the flavonoids with aluminum chloride ($AlCl_3$). The absorbance of the yellow complex formed was measured using a UV-visible spectrophotometer at 415 nm. Quercetin was used as standard.

5. Determination of antioxidant activity

5.1. DPPH radical scavenging assay

The hydrogen atom or electron donation abilities of the extracts were measured from the bleaching of a purple-colored methanol solution of DPPH. This spectrophotometric assay uses the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) as a reagent (Amarowicz et al. 2004). Free radical scavenging activities of ethyl acetate and methanolic extracts of our plants were performed according the method described in El Hajaji's study (El Hajaji et al. 2010). Briefly, a methanol solution of the extract was added to a solution of DPPH (1.01×10^{-4} M), the mixture was sufficiently shaken and allowed to stand in the dark at room temperature for 30 min. The absorbance of the mixture was measured at 519 nm and the result expressed in percentage of inhibition using the formula:

$$\% \text{ inhibition} = [(A_B - A_S)/A_B] \times 100$$

where A_B is the absorbance of the control reaction and A_S the absorbance of test compounds. Butylhydroxytoluene (BHT) was used as control positive. The test was carried out in triplicate.

5.2. Reducing power assay (Iron reducing activity)

The Fe^{3+} reducing power of the extract was determined by the method of Oyaizu (Oyaizu 1986). The concentrations to be tested of each extract are dissolved in the distilled water, and then mixed with phosphate buffer (pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] and then incubated at 50 °C for 20 min. Trichloroacetic acid ($C_2HCl_3O_2$) (10%) was added to the mixture and centrifuged at 3000 rpm for 10 min. Distilled water and ferric chloride ($FeCl_3$)

were mixed with the upper layer of the solution and the absorbance measured at 700 nm. Reducing power was measured by the increasing of absorbance. Reducing power will be important if the absorbance increases. The standards used for reducing power assay were ascorbic acid, tannic acid, and gallic acid. Blank solution was made with phosphate buffer. All analyses were run in triplicate and results averaged.

5.3. Total antioxidant capacity by phosphomolybdenum method

The antioxidant activity of the extract was evaluated by the phosphomolybdenum method according to the procedure described by Prieto (Prieto et al 1999). The test consists of the ability of the extract to reduce the Mo(VI) to Mo(V) and the formation of a green phosphate/Mo(V) complex at acid pH. A 0.3 mL extract was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). Methanol was used in place of extract in the case of blank. The antioxidant capacity was measured using a spectrophotometer by taking the absorbance of the solution at 695 nm. The results are expressed in ascorbic acid equivalent by using the equation given by ascorbic acid used as standard: [$A= 0.0037C + 0.0343$; $R^2= 0.991$], where A is the absorbance at 695 nm and C the concentration as ascorbic acid equivalent (µg/mL). The values are presented as the means of triplicate analysis.

6. Determination of antibacterial activity

6.1. Bacterial strains

Antibacterial activity of the extracts was evaluated against four bacterial strains; two Gram-positive bacteria: *Staphylococcus aureus* ATCC 25922, *Bacillus subtilis* ILP 14283; and two Gram-negative bacteria: *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853.

6.2. Antibacterial assay

The disc diffusion method was used to evaluate the antibacterial activity with some modifications (NCCLS 2012). Briefly, an inoculum of bacterial (100 µL), consisting of 0.5 McFarland (10^7 CFU/mL) in physiologic saline, was inoculated in Petri dishes containing sterile Luria-Bertani Agar medium. Sterile filter paper discs (6 mm diameter) impregnated with 10 µL of each concentration of extract (1, 2, 10, 20, 30, and 40 mg/mL of DMSO at 2%)

were deposited on medium. For the control, discs were impregnated with 10 µL of DMSO at 2%. Each experiment was performed in duplicate.

6.3. Determination of Minimal Inhibitory Concentration (MIC)

The MICs were determined using the method previously described by Bouhdid *et al*. with minor modifications (Bouhdid et al. 2009). 0.15% (w/v) of agar was used as an emulsifier for the broth microdilution assay with resazurin as a bacterial growth indicator. In a polypropylene microplate of 96-well, 50 µL of Luria-Bertani broth were distributed from the second to the 12^{th} well in the first time. Scalar dilutions of 50 µL were transferred from the second to the 11th well. The 12^{th} well was considered as growth control. A final concentration of approximately 107CFU/mL were established by adding 50 µL of a bacterial suspension in each well. As reported in the literature, 10 µL of resazurin were added to each well after incubation of 24 h at 37 °C, for the evaluation of bacterial growth, and a further incubation was done at 37 °C for 2 h (Bouhdid *et al*. 2009). The MIC was determined as the lowest extract concentration that prevented a change in resazurin color, while the reduction of blue dye resazurin to pink resorufin indicated a bacterial growth. Experiments were done in triplicate. After incubation of 24h at 37 °C, the lowest concentration of the extracts yielding negative subcultures will considered as the minimum bactericidal concentration (MBC). It is determined by spotting 2 µL from negative wells on LB plates. Experiments were also done in triplicate.

Results and discussion

1. Phytochemical screening

Many researchers have used early phytochemical analysis to screen plants for their medicinal potential. Phytochemical screening test is of paramount importance in identifying new sources of therapeutically and industrially valuable compounds having medicinal significance, to make the best and judicious use of available natural wealth. The result of phytochemical screening is shown in Table 1. The results revealed the existence of flavonoids and tannins in all extracts except the ethyl acetate extracts of *C. occidentalis* and *T. asiatica.* The absence of alkaloids in all extracts was demonstrated by the absence of precipitates (brownish or yellowish precipitate in the case of Dragendorff test, yellow precipitate in the case of Mayer test, brownish to yellowish precipitate in the case of Wagner test). The presence of phenolic compounds in these plants was also reported in other studies. In fact,

flavonoids and other phenolic compounds were reported in *P. guajava* (Gutierrez et al. 2008; Metwally et al. 2011). In our *P. guajava* extracts, the tests revealed the presence of flavones in ethyl acetate extract and flavanones in methanol extract. Moreover, in both extracts, the tannins are catechic. Recent studies conducted in the aerial parts of *E. hirta* growing in China permitted to isolate nine phenolic compounds (Wu et al. 2012). In leaves, flavonoids and tannins are also shown (Basma et al. 2011). Another study showed the presence of saponins, tannins, cardiac glycosides, alkaloids, anthraquinones, and flavonoids in the entire plant (Abubakar 2009). Flavonoids and tannins contained in the methanolic extract of *C. occidentalis* leaves were also demonstrated in other studies (Nuhu and Aliyu 2008; Silva et al. 2011). Other compounds like anthraquinones, triterpenes, benzophenanthridine alkaloids, cyclohexylamides, coumarins, and less quantity of saponins were also shown (Rajkumar et al. 2008; Silva et al. 2011). In their study, Praveena and Suriyavathana demonstrated the presence of flavonoids in the twigs of *T. asiatica* as observed in our methanolic extract (Praveena and Suriyavathana 2012).

Table 1: Phytochemicals detected in extracts of *P. guajava, C. occidentalis, E. hirta, and T. asiatica.*

Plants	Alcaloids		Flavonoids		Tannins	
	EtOAc	MeOH	EtOAc	MeOH	EtOAc	MeOH
P. guajava	---	---	++-	+++	++-	+++
C. occidentalis	---	---	---	++-	---	+--
E. hirta	---	---	+++	+++	++-	+++
T. asiatica	---	---	---	++-	---	+++

Key: + for present; - for absent

2. Total phenolic and flavonoids contents

Polyphenols are plant secondary metabolites and are essential by virtue of their antioxidant activity by chelating redox-active metal ions, inactivating lipid free radical chains and preventing hydroperoxide conversion into reactive oxyradicals (Vedpriya and Yadav 2011). Table 2 reports the total phenolic compounds and flavonoids in fractions expressed as gallic acid equivalent, and quercetin equivalent respectively. The strong concentration of total phenolic content was observed in *T. asiatica* extracts. It's established at 2.44 g/L GEA for

ethyl acetate extract and 2.21 g/L GEA for methanolic extract. However, less concentration of total phenolic content in ethyl acetate extract was observed in *P. guajava* with 0.65 g/L GEA. However, for methanolic extract, it was observed in *C. occidentalis* with 0.74 g/L GEA. In their study, Venkatachalam et al. (2012), obtained 8 mg GAE/g for the methanolic extract from the leaves of *P. guajava* and 7.38 mg GAE/g for the aqueous extract. As for *E. hirta*, Basma et al. (2011), found the values of 206.17 mg GAE/g and 117.08 mg GAE/g respectively for the leaves and the steams extracts.

About the flavonoids, the results showed that the ethyl acetate extract of *C. occidentalis* had the highest flavonoids content. It's established at 45.33 µg/mL EQ. However, in methanol extract it's observed in *C. occidentalis* with 25.56 µg/mL EQ. Less concentration of total flavonoids content was observed for *E. hirta* for both solvents with 1.26 and 0.36 µg/mL EQ, respectively, for ethyl acetate and methanol extracts.

Table 2: Total phenolic and flavonoid content of *P. guajava, C. occidentalis, E. hirta, and T. asiatica.*

Plants	Total phenols content (g/L GEA)		Total flavonoids content (µg/mL QE)	
	EtOAc	MeOH	EtOAc	MeOH
P. guajava	0.65	1.82	22.03	22.93
C. occidentalis	0.90	0.74	45.33	25.56
E. hirta	2.21	1.30	1.26	0.36
T. asiatica	2.44	2.21	32.03	23.63

3. Antioxidant activity

Antioxidant activity is generally due to different mechanisms such as prevention of chain initiation, decomposition of peroxides, and prevention of continued hydrogen abstraction, free radical scavenging, reducing capacity, and binding of transition metal ion catalysts (Mao et al. 2006) and different methods can be used to evaluate the antioxidant activity (Sarikurkcu et al. 2010). In this study, three antioxidant evaluation methods such as DPPH radical scavenging activity, reducing power assay, and phosphomolybdenum method are used.

3.1. Free radical scavenging activity

Results of the free radical scavenging activity of *P. guajava, T. asiatica, C. occidentalis,* and *E. hirta* are shown in Figures 1 and 2. For ethyl acetate extract, the strong activity was

observed in *E. hirta* with 92% for the highest concentration tested. With the same concentration, *P. guajava* showed a reduction power of 85%. However, with respectively, 36 % and 26%, *T. asiatica* and *C. occidentalis* are manifested the lowest activity (Figure 1). For methanol extract, *E. hirta* was also manifested the strong activity with 92% for the high concentration tested (Figure 2). In the same concentration, *P. guajava* and *T. asiatica* have, respectively, 90% and 68%. With 22%, *C. occidentalis* is also less active against DPPH radicals. In addition, at the lowest concentrations, *P. guajava* is the most active than the other plant extracts.

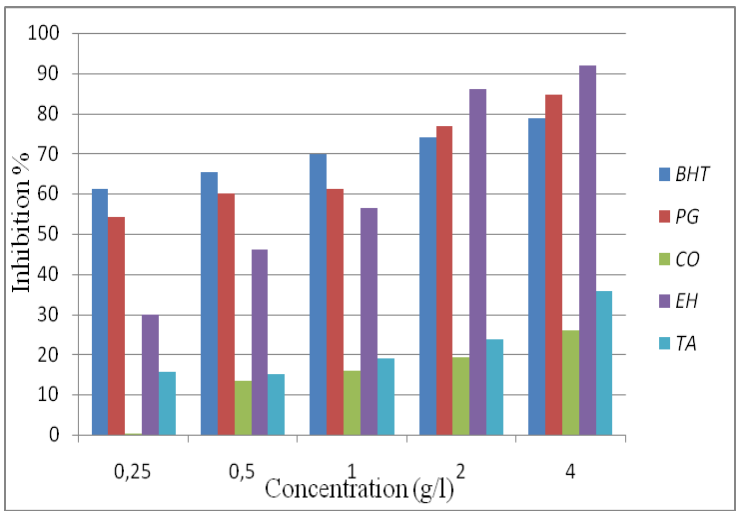

Figure 1: Free radical scavenging activity of ethyl acetate extract of *P. guajava, C. occidentalis, E. hirta,* and *T. asiatica.*

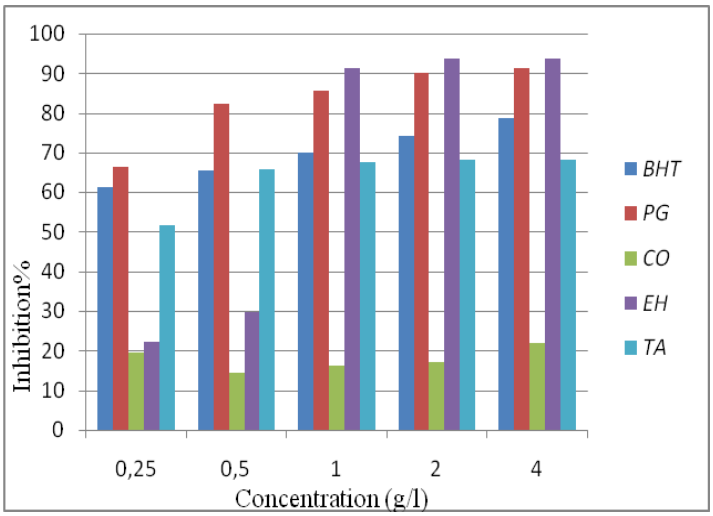

Figure 2: Free radical scavenging activity of methanol extracts of *P. guajava, C. occidentalis, E. hirta* and *T. asiatica.*

The effect of antioxidants on DPPH radical scavenging activity was thought to be due to their hydrogen-donating ability (Shimada et al. 1992). All tested extracts showed an antioxidant activity, which increased with increasing concentration as observed in other studies (Sarikurkcu et al. 2009; Sarikurkcu et al. 2010). A strong correlation was observed between the radical scavenging capacity and polarity of the extracts (Favier 2003). It is also the case for this study. Ethyl acetate and methanol extracts of *P. guajava*, showed a strong activity with IC_{50} values of 0.22 and 0.20 g/L, respectively. The IC_{50} values of all extracts are presented in Table 3. The BHT, a synthetic antioxidant molecule used as control, showed an IC_{50} of 0.2 g/L.

Table 3: Values of IC_{50}.

Plants	IC_{50} (g/L)	
	EtOAc	MeOH
P. guajava	0.22	0.20
C. occidentalis	-	-
E. hirta	0.7	0.68
T. asiatica	-	0.25
BHT	0.20	

3.2. Reducing power assay

The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Uritani et al. 1994). In this study, we used the direct correlation between observed antioxidant activities and reducing power of certain plant extracts to evaluate the possible antioxidant effect of our samples and the results are shown in Figures 3 and 4. For ethyl acetate samples, the strong activity was observed against *E. hirta* with an absorbance of 2.6 nm at the concentration of 1 mg/mL. At the same concentration, *T. asiatica* expressed an absorbance higher than 1 nm (Figure 3). For methanol extract, the greatest activity was also observed in the *E. hirta* extract with the same absorbance (2.6 nm) and concentration (1 mg/mL). For the same concentration, the absorbance of *P. guajava* was 1.2 nm (Figure 4).

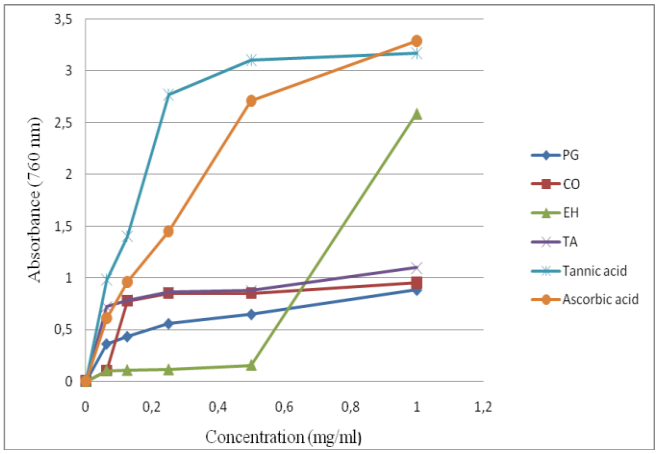

Figure 3: Total reducing power of ethyl acetate extracts of *P. guajava*, *C. occidentalis*, *E. hirta* and *T. asiatica*.

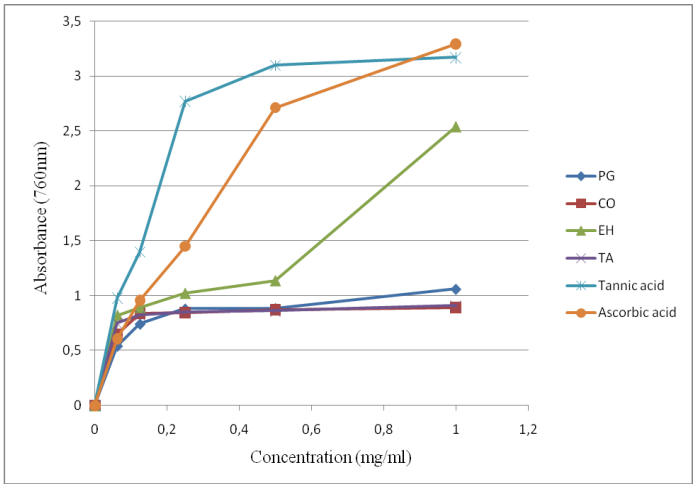

Figure 4: Total reducing power of methanol extracts of *P. guajava*, *C. occidentalis*, *E. hirta* and *T. asiatica*.

3.3. Total antioxidant activity

To evaluate the total antioxidant capacity of our products, we used the phosphomolybdenum method based on the reduction of Mo(VI) to Mo(V) in the presence of antioxidant compounds (Figures 5 and 6). For ethyl acetate extract, the most activity was observed in *T. asiatica* extract with equivalent ascorbic acid concentrations (µg/mL) of 102, 110, and 182 at 25, 50, and 100 µg/mL extract concentrations, respectively. Less activity was manifested by *C. occidentalis* with an equivalent ascorbic acid concentration (µg/mL) respectively of 76 at 25 and 50 µg/mL extract concentrations and 98 at 100 µg/mL. Moreover, for methanol extract, the most activity was observed in *P. guajava* extract with equivalent ascorbic acid concentrations (µg/mL) of 140, 159, and 176 at 25, 50, and 100 µg/mL extract

concentrations, respectively. Less activity was also manifested by *C. occidentalis* with concentrations of 70 at 25 and 50 µg/mL extract concentrations and 90 µg/mL at 100 µg/mL (Figure 6). All extracts showed an antioxidant capacity and manifested a highest compared to that of the gallic acid, taken as antioxidant control.

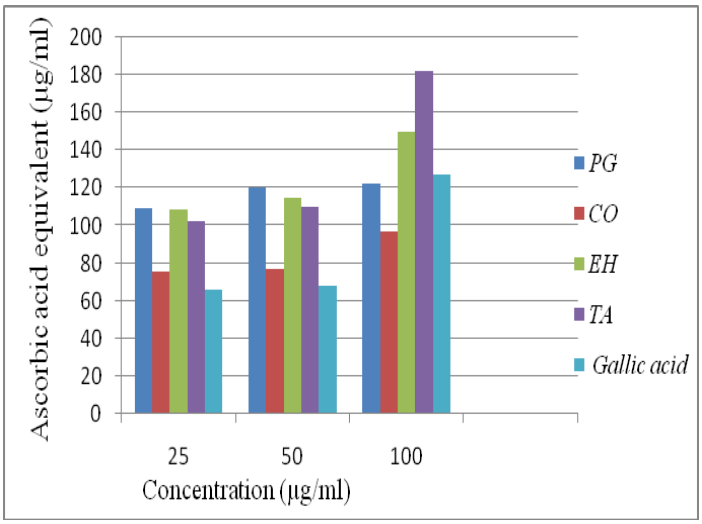

Figure 5: Total antioxidant capacity of ethyl acetate extraacts of *P. guajava*, *C. occidentalis*, *E. hirta* and *T. asiatica.*

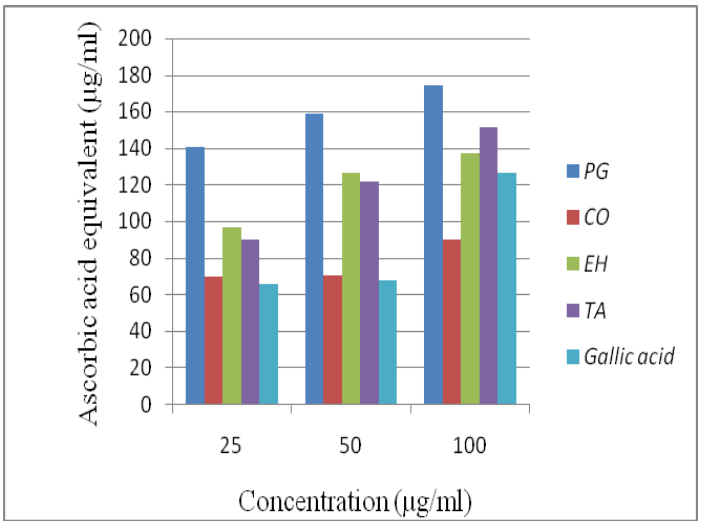

Figure 6: Total antioxidant capacity of methanol extracts of *P. guajava*, *C. occidentalis*, *E. hirta* and *T. asiatica.*

4. Antibacterial activity

The results of the antibacterial activity of methanol extracts are presented in Table 4. The used negative control (DMSO 2%) did not exert any inhibition on the strains tested. Methanol

extracts of the plants exhibited low antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, and Escherichia coli.* However, against *Pseudomonas aeruginosa,* the extracts have not exhibited any antibacterial activity except *E. hirta extract.*

Table 4: Growth inhibition diameters (mm) of the methanolic extract against bacteria.

Bacterial strains	Methanol extracts			
	P. guajava	*C. occidentalis*	*E. hirta*	*T. asiatica*
S. aureus	7	8	10	9
B. subtilis	7	8	9	10
E. coli	8	7	8	7
P. aeruginosa	-	-	7	-

4.1. Minimal inhibitory and bactericidal concentrations

Minimal Inhibitory and Bactericidal Concentrations are shown in Table 5. The extracts showed very low or no antibacterial activity against all strains tested. Accordingly, no minimal inhibitory concentration has been determined.

Table 5: Determination of MBC values of the methanolic extracts against *S. aureus, S. subtilis, E. coli* and *P. aeruginosa.*

Concentrations (mg/mL)	Methanol extracts of *P. guajava, C. occidentalis, E. hirta, and T. asiatica*			
	S. aureus ATCC 25922	*B. subtilis* ILP 14283	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853
Control	+	+	+	+
50	+	+	+	+
25	+	+	+	+
12.5	+	+	+	+
6.25	+	+	+	+
3.125	+	+	+	+
1.5625	+	+	+	+
0.78125	+	+	+	+
0.390625	+	+	+	+
0.1953125	+	+	+	+
0.09765625	+	+	+	+
0.048828125	+	+	+	+

Key: + for presence of growth; - for absence of growth.

In Comorian traditional medicine, *P. guajava*, *C. occidentalis*, *E. hirta,* and *T. asiatica* are largely used against infectious diseases and bacterial diseases (Soulé et al. 2014). It is also known that the biological activities of natural compounds are mainly due to the presence of polyphenolic compounds. The methanolic extract of *P. guajava*, *C. occidentalis*, *E. hirta,* and *T. asiatica* contained flavonoids, tannins, and polyphenolic compounds (Table 1). The methanol extract should show a good antibacterial effect; however, as observed in Tables 4 and 5, very low or even any activity manifested. In others studies antibacterial effects of *E. hirta* (Perumal and Mahmud, 2013; Mussadique Hussain et al., 2014), *P. guajava* (Ngene et al., 2019), and *T. asiatica* (Duraipandiyan and Ignacimuthu, 2009) were demonstrated. Our results might be explained by the possible alteration of compounds due to the conjugated action of temperature and the time of passage in Soxhlet apparatus. In effect, the antibacterial activities observed in *E. Hirta*, *T. Asiatica* and *P. Guajava* come from cold-made extracts (maceration) while ours are hot extracts (Soxhlet extracts).

Conclusions

Pytochemical compounds, total phenolic and flavonoids contents, and antioxidant capacities of *P. guajava*, *T. asiatica*, *C. occidentalis* and *E. hirta* extracts were determined. Phytochemical screening revealed the presence of flavonoids and tannins in all extracts except the ethyl acetate extracts of *C. occidentalis* and *T. asiatica*. Total phenolic and flavonoids contents results showed a large dominance of *T. asiatica* extracts in phenol content and ethyl acetate extract of *C. occidentalis* for total flavonoids content. For antioxidant activity, all tests showed significant dose-dependent antioxidant activity. For DPPH radical scavenging activity and reducing power assay, it's the *E. hirta* extracts, which manifested the highest activity. However, for total antioxidant capacity, the stronger activity was manifested by *P. guajava* extracts and ethyl acetate extract of *T. asiatica*. *P. guajava, C. occidentalis, E. hirta,* and *T. asiatica* methanol extracts exhibit low antibacterial activity. By its no solubility in DMSO, ethyl acetate extracts could not test against bacterial strains. Indeed, the temperature and time of passage of compounds in Soxhlet apparatus probably altered these compounds and affected the antibacterial effect of methanol extract. Therefore, in the future, we must extract these compounds under different experimental conditions for the antibacterial tests. The results suggested that *P. guajava, C. occidentalis, T. asiatica* leaves, and *E. hirta* aerial part can be considered as good sources of natural antioxidants. It is

then necessary to identify and isolate the compounds that are responsible for these antioxidant activities.

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Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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