

Endemic Asteraceae from Mauritius Islands as Potential Phytomedicines

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Abstract—The present study aimed at validating the phytomedicinal properties of selected species from the Asteraceae family, namely *Psiadia arguta* (Pa), *Psiadia viscosa* (Pv) *Psiadia lithospermifolia* (Pl) , and *Distephanus populifolius* (Dp). Hexane, ethyl acetate, and methanol leaf extracts were studied for their antioxidant properties and antibacterial activity. The phenolics levels varied from 24.05 to 231.6 mg gallic acid equivalent/g with the maximum level in methanol extracts of Pa and Dp. The highest flavonoids and proanthocyanidins content were noted in Pa methanolic extract with 65.7 mg quercetin/g and 25.05 mg catechin/g extract, respectively. Pa polar extracts had also the maximum free radical scavenging activity(DPPH) with IC₅₀ 11.31 and 11.6 µg/ml respectively. The maximum ferric reducing potential(FRAP) was noted in Pl methanol extracts 281.60 mmol Fe²⁺/g extract. The antioxidant capacity based on DPPH and FRAP values were positively correlated to total phenolics, flavonoid and proanthocyanidins. All four species exhibited antimicrobial activity against the tested bacteria (both Gram-negative and Gram-positive). Hexane and ethyl acetate extracts of *Psiadia* species were the most promising extracts, with the lowest minimum inhibitory concentrations (MIC) values ranging from 19.53 to 78.125 µg/ml.

Keywords— antibacterial, DPPH, flavonoids, FRAP, *Psiadia* spp.

I. INTRODUCTION

Medicinal plants are important sources of biologically active compounds which can be used for drug development. Currently, research is geared towards the anti oxidant activity of plant extracts due to diseases caused by oxidative stress [1]. Additionally, antibiotic resistance continues to be a major concern globally. The Asteraceae (sun flower family) is one of the largest families of flowering plants with over 1000 genera and 25000 species. Many plants species from this family are reputed for their medicinal properties and enter the local pharmacopeias of countries. Moreover, the Asteraceae is abundantly rich in sesquiterpene lactones (SLs) which is a class of naturally occurring plant terpenoids, which might contribute to the medicinal properties of the Asteraceae plants [2][3]. Some endemic Asteraceae of Mauritius include *Psiadia arguta* (Pa) *Psiadia lithospermifolia* (Pl) *Psiadia*

viscosa (Pv) and *Distephanus populifolius* (Dp). In the Mauritian folk medicine, leaves of *Psiadia* plants are usually employed to treat cutaneous infections, as herbal teas against bronchial infections and also to release stress [4]. To our knowledge, no investigations have previously been carried out using pharmacological assays to endorse the medicinal properties of the selected species from Asteraceae family. The findings hereunder validate the use of these selected Asteraceae in the traditional medicine of Mauritius and also highlight their pharmaceutical potential as prospective phytomedicines.

II. PROCEDURE

A. Plant material and extraction procedure

The leaves from the four Asteraceae species were collected from Arboretum Robinson Curepipe Mauritius. Voucher specimens were deposited at the Mauritius Herbarium, Ministry of Agro-Industry and Food security Réduit thereby accession numbers were given as follows: *P. arguta* 26407; *P. lithospermifolia* 26404, *P. viscosa* 26406 and *D. populifolius* 26405. The leaves (150g) of all four species were sequentially extracted using cold hexane, the marc were further extracted with cold ethyl acetate and methanol solvent Finally, the solvent from each filtrate solvent was concentrated in a rotary evaporator under reduced pressure and low temperature.

B. Qualitative analysis

Phytochemical screening was conducted according to [5][6][7] .

C. Quantitative analysis

Determination of total Phenolic content

Total phenolic content of organic extracts was determined by Folin–Ciocalteu method by adapting the procedures of [8].

Determination of total flavonoid content

Total flavonoid content of organic extracts was determined by the aluminum chloride colorimetric method [9].

Determination of proanthocyanidins

Total proanthocyanidins content of organic extracts was carried out as recommended by [10] . A calibration curve was prepared using catechin.

D. Anti oxidant activity

DPPH free-radical scavenging activity

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The radical scavenging effect of different solvent extracts was studied by DPPH free radical scavenging assay according to the methods of [11]

Ferric Reducing antioxidant power (FRAP)

The reducing ability of different solvent extracts was determined according to [12]. Results are expressed in mM Fe (II)/g

E. Anti bacterial assay

The serial dilution technique as described by [13] was used to determine the minimum inhibitory concentration (MIC) for antibacterial activity of hexane, ethyl acetate and methanol extracts. Six bacterial strains, *Escherichia coli* (ATCC 27853), *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212), *Klebsiella pneumoniae* (ATCC27853), *Pseudomonas aeruginosa* (ATCC 27853) and *Bacillus cereus* (ATCC 11778) were used.

F. Statistical analysis

All experiments were conducted in triplicate. For the MIC values, re-evaluation of the growth inhibition was conducted. Statistical analysis was performed using one way ANOVA and results were compared using the Turkey test at a 5% significance level using Minitab 16. IC₅₀ was computed using Origin Pro and Pearson correlation was calculated on excel.

III. RESULT

A. Phytochemical screening

Studied endemic plants expose themselves as a source of coumarins, tannins, alkaloids and terpenoids. Coumarins were detected in ethyl acetate and methanol extracts of Pl. On the other hand, tannins were present in methanol extracts of all the studied extracts. The Dragendorff's reagent showed the presence of alkaloids in trace amount only in ethyl acetate extracts of the species and exceptionally trace amount of alkaloids was detected in hexane extract of Dp only. Finally, terpenoids were present in both hexane and ethyl acetate extracts of all the species.

B. Total phenol contents (TPC), total flavonoid contents (TFC) and Total proanthocyanidin content (TPC).

Total phenolic content of the different extracts namely hexane, ethyl acetate and methanol of studied species was solvent dependent and expressed as milligrams of gallic acid equivalents gram per extract. Fig 1 summarises that total phenolic amounts in extracts varied widely, ranging from 24.05 to 231.7 mg gallic acid/g of extracts. Methanol extract of Pa exhibited the highest total phenolic content. The flavonoid content expressed as milligrams quercetin equivalents gram per extract varied from 1.48 to 65.7 mg quercetin/g extract (Fig 2) There were significant differences between methanol extracts of Pv and methanol extracts of Pa,Pl and Dp. The proanthocyanidin content expressed as milligrams catechin equivalents gram per extract varied from 25.05 to 15.1 mg catechin /g extract (Fig 3).

C. Free-Radical Scavenging activity assay.

Free radical scavenging capacities of the tested extracts was measured by the DPPH assay. Extracts exhibiting 50% inhibition as shown in Fig 4 were selected to determine their IC₅₀. Among the extracts methanol and ethyl acetate extracts of Pa and methanol extracts of Dp showed significant ($p < 0.05$) free radical scavenging activity with percentage inhibition 92.11% 92.87% and 92.90% respectively. The IC₅₀ of methanol and ethyl acetate extracts of Pa (11.31 and 11.6 µg/ml) and methanol extract of Dp (11.27 µg/ml) were significantly similar to ascorbic acid standard (10.04 ± 0.02 µg/ml).

D. Ferric reducing antioxidant power (FRAP) assay

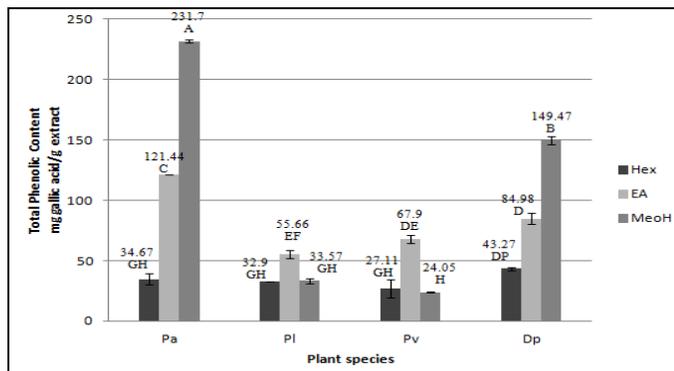
In the current study, all tested extracts manifest anti-oxidant ability as shown in Table I. Hexane, ethyl acetate and methanol extract exhibited ferric reducing capacity. However, the activity varied widely from hexane extract of Dp 96.26 to methanol extract of Pl 281.60 mmol Fe²⁺/g extract. Methanol extracts of Pa, Pl, Dp (275.19, 281.60 and 206.91 mmol Fe²⁺/g extract) demonstrated significantly ($p < 0.05$) high ferric reducing capacity.

E. Anti bacterial activity

The MIC values of the hexane, ethyl acetate and methanol extracts of endemic plants using microdilution assay are indicated in Table II the results demonstrate that all four endemic plants species exhibited varying degrees of activity against the tested bacterial species. The extracts also appeared to be broad spectrum as their activities were independent on the gram reaction. It was generally noted that the hexane and ethyl acetate extract of *Psiadia sp* were significantly more active (MIC: 19.53 to 78.12) µg/ml against all the three gram negative and gram positive bacteria than methanol extracts. Promising activity was noted with Pa, Pl and Pv hexane and ethyl acetate extracts against *Escherichia coli* at a concentration of 19.53 µg/ml. The anti bacterial activity of Pa hexane and ethyl acetate extracts and Pl hexane extract was significantly high against *Klebsiella pneumoniae* at concentration of 19.53 µg/ml. Wells containing methanol as negative control showed no colour change.

F. Correlation analysis

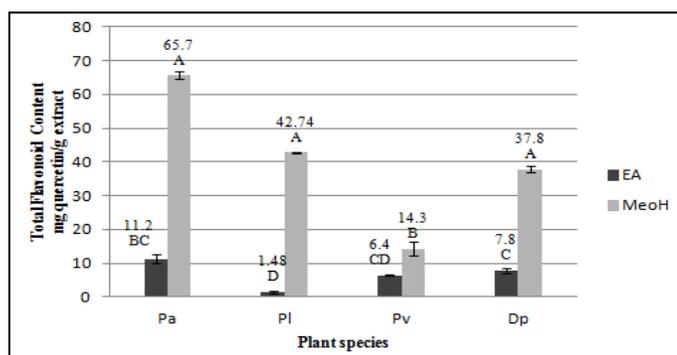
The DPPH and FRAP strongly correlated with the total phenolics, flavonoids and proanthocyanidins content. The DPPH activity of methanol extracts positively correlates with total phenol ($r=0.94$), flavonoid ($r=0.65$) and proanthocyanidin ($r=0.46$). On the other hand, the FRAP activity of methanol and ethyl acetate extract correlates positively with total phenol (M; $R=0.47$, EA; 0.95), flavonoid (M; $R=0.88$ EA; $R=0.98$) and proanthocyanidin for methanolic extracts ($R=0.95$).



Hex. Hexane ;EA. Ethyl Acetate ;MeOH. Methanol

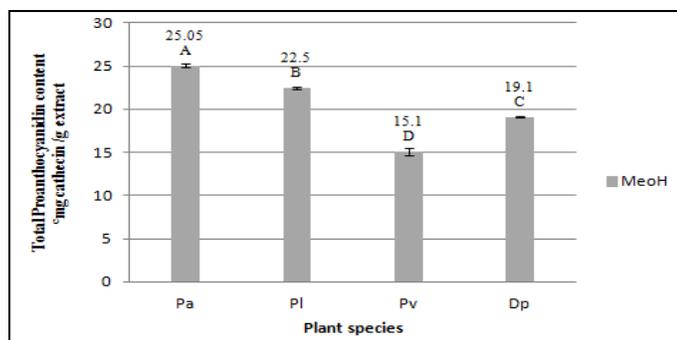
Different letters between bars represent significant difference between samples (p<0.05)

Fig 1 Total Phenolic content of extracts of endemic Asteraceae from Mauritius.



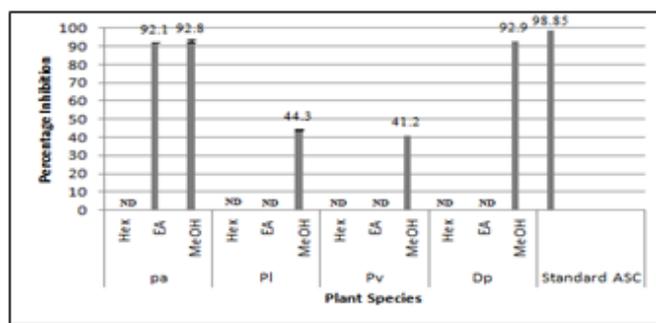
Different letters between bars represent significant difference between samples (p<0.05). EA. Ethyl acetate; MeOH.: Methanol

Fig. 2 Total Flavonoid content of leaf extracts of endemic Asteraceae from Mauritius.



Different letters between bars represent significant difference between samples (p<0.05). MeOH. Methanol

Fig. 3 Total Proanthocyanidin content of endemic leaf extracts of endemic Asteraceae from Mauritius.



Hex Hexane, EA :Ethyl acetate; MeOH methanol ASC Ascorbic acid, ND Not detected.

Fig 4 DPPH Free radical scavenging activity of leaf extracts of endemic Asteraceae from Mauritius.

TABLE I
FERRIC REDUCING ABILITY OF LEAF EXTRACTS OF ENDEMIC ASTERACEAE FROM MAURITIUS.

Hex: Hexane, EA :Ethyl Acetate ;MeOH :Methanol

Sample	FRAP mmol Fe2+/g extract		
	Hex	EA	Me
Pa	195.87 bc	184.57 c	275.19 a
Pl	115.01 e	107.05 ef	281.60 a
Pv	144.01 d	143.75 d	94.47f
Dp	96.26 f	147.86 d	206.91b

IV. DISCUSSION

Phytochemical screening of plants is aimed at revealing the qualitative chemical composition of the extracts. In the above study, we showed that endemic plants extracts of the Asteraceae endemic to Mauritius are important sources of secondary metabolites such as coumarins, tannins, alkaloids and terpenoids. These compounds are considered to be medicinally important to prevent and treat diseases [14]. So far, no previous research has been reported on the phytochemical profile of hexane, ethyl acetate and methanolic extracts of selected *Psiadia* and *Distephanus sp.* The presence of coumarins in the *Psiadia sp* was surprising since there exists only one report on the isolation of coumarins (7-prenyloxycoumarin) from *Psiadia dentata* endemic to Reunion Islands [15]. In the present work, tannins were detected in in methanol extracts of all the species. The results of this work corroborate with the work of [16] whereby considerable amount of tannins were found in methanolic extracts of selected Asteraceae plants. On the other hand, only traces of alkaloids were detected in the above species which was predictable since the occurrence of alkaloids in *Psiadia sp* and *Distephanus sp* are rare in literature though well reported in other genera of Asteraceae family [17]. Terpenoids have also been identified in the less polar extracts of the screened Asteraceae species. These findings agree with those of Govinden Soulange et al 2004[3], whereby the occurrence of (*E*)-isoasarone, pentyl-4-(1-methylethyl benzoate), (*Z*)-isoasarone and isoeugenol were reported in essential oil

obtained from endemic *Psiadia* species in Mauritius. Moreover, [18] isolated and characterised two different diterpenoids from the exudates of *Psiadia punctulata* from Africa. It is anticipated that the medicinal properties of studied species can be linked to the presence of phytochemicals identified.

In order to determine the recovery of polyphenolic content the leaf sample was extracted using three different solvents polarity (hexane, ethyl acetate and methanol). The amount of

TABLE II
ANTIBACTERIAL ACTIVITY OF ASTERACEAE LEAF EXTRACTS

Sample	MIC value $\mu\text{g/ml}$								
	<i>Klebsiella pneumoniae</i>			<i>Pseudomonas aeruginosa</i>			<i>Escherichia coli</i>		
	Hex	EA	Me	Hex	EA	Me	Hex	EA	Me
Pa	19.53	19.53	156.25	78.125	78.125	39.06	19.53	19.53	156.25
Pl	19.53	39.06	625.0	19.53	78.125	625	19.53	19.53	312.5
Pv	78.12	19.53	156.25	78.125	39.06	156.25	19.53	19.53	156.25
Dp	625	156.25	312.5	39.06	39.06	39.06	312.5	39.06	78.125
+ve control	15.63			15.63			62.5		
Sample	<i>Staphylococcus aureus</i>			<i>Enterococcus faecalis</i>			<i>Bacillus cereus</i>		
	Hex	EA	Me	Hex	EA	Me	Hex	EA	Me
	Pa	19.53	19.53	78.125	19.53	19.53	1250	19.53	19.53
Pl	19.53	19.53	78.125	19.53	19.53	1250	19.53	39.06	78.125
Pv	19.53	19.53	312.5	19.53	19.53	1250	39.06	39.06	78.125
Dp	312.5	78.125	156.25	156.25	19.53	19.53	625	312.5	312.5
+ve control	15.62			3.91			31.25		

MIC: Minimum Inhibitory Concentration: $\mu\text{g/ml}$; Hex: Hexane, Ea: Ethyl acetate and Me: methanol

phenolics varied in different extracts. However, highest total phenol content was recorded in methanol extracts and ethyl acetate extracts of Pa (M: 231.70, EA: 121.44 GEA/g), DP (M: 149.70, EA: 84.98 GEA/g) Fig1. On the other hand, total flavonoid content were significantly high in methanol extracts of Pa, Pl and Dp ($P > 0.05$) (M: 65.7 M: 42.74 M: 37.8 QE/g) respectively Fig2. Total proanthocyanidin content was highest in methanol extracts of Pa and Pl (25.05, 22.5 CAT/g) Fig 3. Generally it was noted that methanol and ethyl acetate extracts was better solvents than hexane for extracting phenolic compounds owing to their higher polarity and good solubility [19]. The determined phenolic content of *Psiadia sp* agreed with the previous study conducted by [20] whereby 5 phenolic compounds namely kaemferol-3-methyl ether, quercetin-3-methyl ether, 3-caffeoyl quinic acid and 3,4-O-dicaffeoyl quinic acid was isolated from *Psiadia terebinthina* endemic to Mauritius island. Hence the presence of these phenolic compounds might explain the consequent level of total phenolic content in Pa, Pl and DP plants. The finding of this study indicates polyphenolic richness of endemic Asteraceae of Mauritius.

Epidemiological studies strongly support the contribution of polyphenols to the prevention of many diseases such as cancers and neurodegenerative diseases [21]. This is the first study to report the antioxidant activity of selected *Psiadia* and *Distephaus sp*. Interestingly, in the present work there was a positive correlation between antioxidant profile 1 and of phenolic content. The results clearly indicate that extracts that contained high total phenolic, flavonoid and proanthocyanidin content showed relatively high antioxidant activity whereby methanol and ethyl acetate extracts of Pa (IC_{50} M: 11.31, EA 11.6 $\mu\text{g/ml}$) and methanol extract of Dp (IC_{50} M: 11.27 $\mu\text{g/ml}$) showed promising radical scavenging activity which were

significantly comparable to standard ascorbic acid (IC_{50} 10.04 $\mu\text{g/ml}$). Similar trend was noted in the FRAP assay (Table I) whereby the methanol extracts of Pa, Pl and Dp exhibited strong reducing power 275.19, 281.60 and 206.91 FRAP mmol Fe^{2+}/g extract respectively. Positive association between phenolic content and antioxidant activity has also been reported in tea, wines and fruit juices [22][23]. Moreover, the present finding reveals that the antioxidant capacities of the Mauritian endemic medicinal plants studied were much higher than many commonly used medicinal plants cited in the literature. For instance, study of the free radical scavenging activity on 4 Southern African medicinal Plants, [24] reported IC_{50} values of the methanol extract between 850 and 2335 $\mu\text{g/ml}$. Investigating the antioxidant potential of 56 selected Chinese medicinal plants, [25] observed that the FRAP values varied between 0.15 and 856.92 $\mu\text{mol Fe}^{2+}/\text{g}$.

The MIC values of the leaf extracts of four species of the Asteraceae family are presented in Table II. Extracts had a broad spectrum of activity against all bacterial strains. According to [26] plants extracts with an MIC value less than 100 $\mu\text{g/ml}$ are usually considered as significantly active and are regarded as promising candidates for further pharmacological studies. In this study, significant activities were recorded with hexane and ethyl acetate extracts of Pa, Pl and Pv with MICs ranging from 19.53 and 78.125 $\mu\text{g/ml}$ respectively. This indicates that the antimicrobial compounds of hexane and ethyl acetate extract of *Psiadia sp* are non-polar and or semi polar in nature. As noted above, the phytochemical profiling of hexane and ethyl acetate extracts of Pa, Pl and Pv plants revealed the presence of considerable amount of terpenoids. Interestingly, terpenoids and essential

oils are commonly reported for their antibacterial properties [27]. Accordingly, essential oil from PI was reported by [3] to significantly inhibit the growth of *Bacillus cereus*, *Staphylococcus aureus* and *Pseudomonas aureofaciens*, *Aspergillus ochraceus*, *Candida pseudotropicalis*, *Kluyveromyces lactis* and *Fusarium moniliforme*.

V. CONCLUSION

Mauritian endemic Asteraceae plants, particularly the species Pa and Dp methanolic extracts, contain significant levels of phenolic compounds and exhibit considerable antioxidant activity. Moreover, the *Psiadia* species (Pa, PI and Pv) hexane and ethyl acetate extracts reveal promising antibacterial activity whereby they can be considered as potential sources of antimicrobial agent worth of pharmacological exploitation.

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