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Pharmacological properties and chemical profiles of *Passiflora foetida* L. extracts: Novel insights for pharmaceuticals and nutraceuticals

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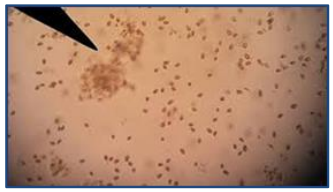
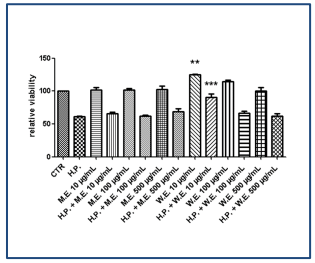
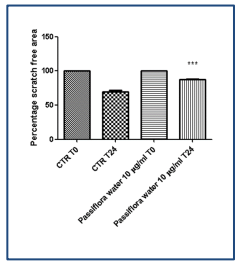
***Artemia salina* lethality bioassay, Hypo-E22 cell viability and wound healing test**



LC_{50} > 5 mg/mL





Passiflora foetida L. extracts

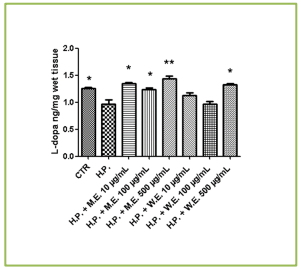
Extracts	TPC (mg GAE/g)	TFC (mg RE/g)
EA	39.61 ± 0.26 [a]	14.50 ± 0.54 [b]
MeOH	24.59 ± 0.24 [b]	10.52 ± 0.59 [c]
MeOH (80%)	21.30 ± 0.20 [d]	50.11 ± 0.78 [a]
Water maceration	22.18 ± 0.16 [c]	3.22 ± 0.45 [d]
Infusion	24.24 ± 0.51 [b]	4.27 ± 0.45 [d]

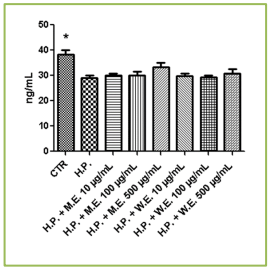
Phytochemical profile, antioxidant abilities and enzyme inhibitory properties

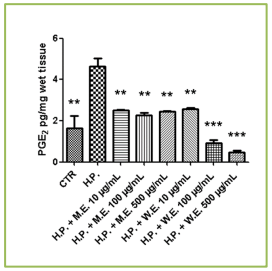
Extracts	DPPH	ABTS	CUPRAC	FRAP	Metal Chelating	PBD
	(mg TE/g)				(mg EDTAE/g)	(mM TE/g)
EA	26.69 ± 1.85 [b]	68.98 ± 2.80 [a]	162.83 ± 0.24 [a]	65.35 ± 0.57 [a]	15.60 ± 0.26 [c]	2.68 ± 0.19 [a]
MeOH	28.57 ± 0.36 [b]	50.99 ± 0.89 [c]	103.00 ± 1.93 [b]	35.76 ± 1.43 [b]	19.21 ± 0.09 [a]	2.28 ± 0.11 [b]
MeOH (80%)	31.74 ± 0.90 [a]	55.06 ± 3.32 [c]	66.83 ± 0.21 [c]	30.36 ± 0.48 [d]	15.24 ± 0.58 [c]	0.66 ± 0.10 [d]
Water maceration	20.77 ± 0.18 [c]	61.81 ± 1.44 [b]	54.46 ± 1.44 [e]	31.59 ± 0.28 [c,d]	14.10 ± 0.40 [d]	1.01 ± 0.01 [c]
Infusion	21.81 ± 0.37 [c]	62.08 ± 1.11 [b]	61.33 ± 1.70 [d]	32.38 ± 0.08[c]	18.00 ± 0.12 [b]	1.02 ± 0.02 [c]

Extracts	AChE	BChE	Tyrosinase	Amylase	Glucosidase
	(mg GALAE/g)		(mg KAE/g)	(mM ACAE/g)	
EA	na	2.45 ± 0.18	48.48 ± 3.68 [a]	0.59 ± 0.01 [a]	0.70 ± 0.01 [a]
MeOH	2.22 ± 0.04 [a]	na	29.33 ± 1.71 [b]	0.43 ± 0.03 [b]	0.37 ± 0.01 [b]
MeOH (80%)	0.92 ± 0.02 [b]	na	35.11 ± 5.77 [b]	0.35 ± 0.02 [c]	0.30 ± 0.01 [c]
Water maceration	0.77 ± 0.08 [c]	na	na	0.17 ± 0.01 [d]	0.11 ± 0.01 [d]
Infusion	0.50 ± 0.08 [d]	na	na	0.41 ± 0.01 [b]	0.28 ± 0.01 [c]

Neuroprotective effects and antimicrobial activity







Dermatophytes (ID strain) A3:D15	Minimum Inhibitory Concentration (MIC)		
	Methaol Extract (µg mL^{-1}) *	Water Extract (µg mL^{-1}) *	Griseofulvin (µg mL^{-1}) *
Arthroderma crocatum (CCF 5300)	12.4 (7.81–15.625)	39.37(31.25–62.5)	>8
Arthroderma curreyi (CCF 5207)	6.19 (3.9–7.81)	12.4(7.81–15.625)	>8
Arthroderma gypseum (CCF 6261)	157.49 (125–250)	396(250–500)	1.587(1–2)
Arthroderma insingulare (CCF 8417)	12.4 (7.81–15.625)	19.68(15.625–31.25)	>8
Arthroderma quadrifidum (CCF 5792)	78.74 (62.5–125)	314.98(250–500)	>8
Trichophyton mentagrophytes (CCF 4823)	49.6 (31.25–62.5)	99.21(62.5–125)	2.52(2–4)
Trichophyton mentagrophytes (CCF 5930)	157.49 (125–250)	396(250–500)	3.174(2–4)
Trichophyton rubrum (CCF 4933)	39.37 (31.25–62.5)	99.21(62.5–125)	1.26(1–2)
Trichophyton rubrum (CCF 4879)	99.21 (62.5–125)	198.42(125–250)	3.175(2–4)
Trichophyton tonsurans (CCF 4834)	9.84 (7.81–15.625)	9.84(7.81–15.625)	0.198(0.125–0.25)

Abstract:

In the present study, *Passiflora foetida* extracts characterized by different polarities were studied for their phytochemical profile, enzyme inhibitory, and antioxidant potentials. *In vitro* and *ex vivo* studies were also carried out on methanol and water extracts for predicting pharmacokinetics and pharmacodynamics. In this regard, neuronal HypoE22 cells, isolated mouse skin tissues, and pathogen dermatophytes strains were exposed to extracts. Emphasis was given to the preventing effects induced by the extracts on hydrogen peroxide-induced alterations of prostaglandinE_2 (PGE2), l-dopa, and serotonin. Chemical analysis revealed the presence of similar compounds in infusion and methanolic extracts. The *ex vivo* studies also showed protective skin properties by *P. foetida* water and methanol extracts, as evidenced by the decrease of hydrogen peroxide-induced PGE_2 level. Additionally, the blunting effects on hydrogen peroxide-induced l-dopa levels are consistent with the anti-tyrosinase effect exerted by both extracts. Finally, microbiological tests demonstrated the efficacy of *P. foetida* methanol and water extracts as anti-mycotic agents against *Trichophyton* and *Arthroderma* species, involved in skin inflammation. Hence, *P. foetida* L. extracts could represent potential sources of pharmaceuticals and nutraceuticals.

Keywords: *Passiflora foetida*; chemical profile; anti-oxidant/anti-inflammatory effects; neuroprotection;skin protection.

Introduction

Passiflora foetida L. popularly known as striking passionflower is a particularly renowned species belonging to the genus *Passiflora*, with tremendous ethnobotanical applications. For instance, the decoction of leaves and fruits of *P. foetida* has been reported to treat asthma and biliousness, while the leaves and root decoction is employed as an emmenagogue and used in hysteria. Additionally, leaf paste is applied to the head for headache and giddiness. Besides, the herb is used in the form of poultices or lotions for erysipelas and skin diseases with inflammation. *P. foetida* has also been described to treat anxiety, insomnia, sexual dysfunction, convulsion, cough as well as cancer. Moreover, studies conducted on *P. foetida* have revealed extracts of the plant to possess numerous promising bioactivities such as antidiarrhoeal, antiulcerogenic, analgesic, antidepressant, anti-inflammatory, anti-hypertensive, hepaprotective, anticancer, antibacterial and antinociceptive. Although the existence of extensive documentation on the traditional uses of *Passiflora* species associated with a variety of health benefits, many species of the genus have still remained underexplored.

Therefore, phytochemical profile, antioxidant, and enzyme inhibition activities were studied and *P. foetida* extracts characterized by different polarities were analyzed. *In vitro* and *ex vivo* studies were also carried out on methanol and water extracts in order to predict pharmacokinetics and putative targets underlying traditional and innovative pharmacological applications of *P. foetida.* In this regard, the multidirectional pharmacological approach focused on the activities of the extracts as protective agents, on neuronal HypoE22 cells and isolated skin tissues, and antimicrobials against selected dermatophyte strains deeply involved in skin inflammation. Emphasis was given to the preventing effects induced by the extracts on the alterations of prostaglandin E_2 (PGE_2), l-dopa, and serotonin levels following oxidative stress stimulus (hydrogen peroxide) challenging.

Results and discussion-Profile of Bioactive Compounds

Total phenolic and flavonoid contents of the tested extracts

Extracts	TPC (mg GAE/g)	TFC (mg RE/g)
EA	39.61±0.26	14.50±0.54
MeOH	24.59±0.24	10.52±0.59
MeOH (80%)	21.30±0.20	50.11±0.78
Water maceration	22.18±0.16	3.22±0.45
Infusion	24.24±0.51	4.27±0.45

Values are reported as mean±S.D. EA: Ethyl acetate; MeOH: methanolic; TPC: Total phenolic content; TFC: Total flavonoid content; GAE: Gallic acid equivalent; RE: Rutin equivalent

- Ethyl acetate extract contained the highest phenolic content, the methanolic extract (80%) was found to yield the highest flavonoid content compared to the other extracts. Water maceration and infusion extracts contained the lowest TFC
- The phytochemical analysis of methanolic and infusion extracts revealed the same 47 compounds to be present in both extracts
- Methanolic extract was found to contain an additional compound, namely isorhamnetin-O-hexoside
- The extracts were found to contain miscellaneous mixtures of compounds composed of flavones, flavonols, and their derivatives
- The chemical profiles of extracts of *P. foetida* identified in the present study suggest that the plant can be a good source of flavonoids

Results and discussion-Antioxidant abilities

Antioxidant abilities of the tested extracts

Extracts	DPPH	ABTS	CUPRAC	FRAP	Metal chelating	PBD
	(mg TE/g)				(mg EDTAE/g)	(mM TE/g)
EA	26.69±1.85	68.98±2.80	162.83±0.24	65.35±0.57	15.60±0.26	2.68±0.19
MeOH	28.57±0.36	50.99±0.89	103.00±1.93	35.76±1.43	19.21±0.09	2.28±0.11
MeOH (80%)	31.74±0.90	55.06±3.32	66.83±0.21	30.36±0.48	15.24±0.58	0.66±0.10
Water maceration	20.77±0.18	61.81±1.44	54.46±1.44	31.59±0.28	14.10±0.40	1.01±0.01
Infusion	21.81±0.37	62.08±1.11	61.33±1.70	32.38±0.08	18.00±0.12	1.02±0.02

Values are reported as mean±S.D. EA: Ethyl acetate; MeOH: methanolic; TE: Trolox equivalent; EDTAE: EDTA equivalent; PBD: Phosphomolybdenum. Different letters (a, b, c, d, and e) indicate significant differences in the extracts ($p < 0.05$)

- All tested extracts were found to be fairly good radical scavengers
- In DPPH assay, the methanolic (80%) extract showed the highest scavenging potential
- In ABTS, CUPRAC and FRAP assays, the ethyl acetate extract was the most potent
- All extracts acted as metal chelators, although the highest metal chelating effects were achieved by methanolic and infusion extracts
- Regarding the phosphomolybdenum assay, ethyl acetate extract showed the highest while methanolic (80%) extract the lowest activity

Results and discussion-Enzyme inhibitory properties

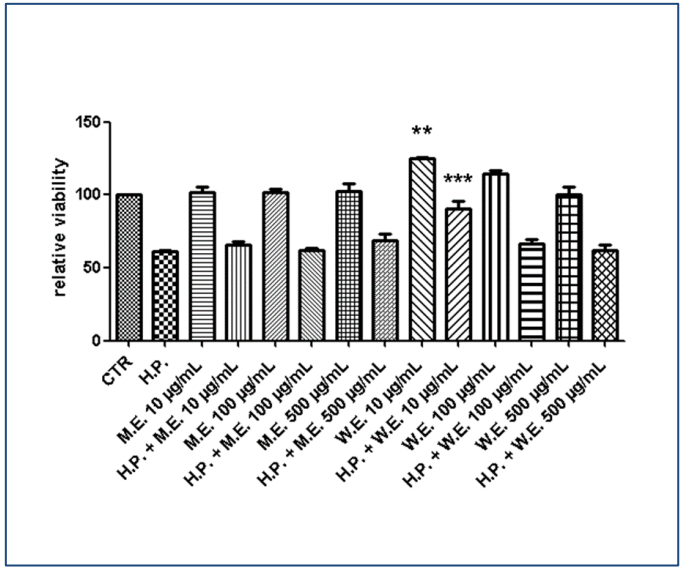
Enzyme inhibitory properties of the tested extracts

Extracts	AChE	BChE	Tyrosinase	Amylase	Glucosidase
	(mg GALAE/g)		(mg KAE/g)	(mM ACAE/g)	
EA	na	2.45±0.18	48.48±3.68	0.59±0.01	0.70±0.01
MeOH	2.22±0.04	na	29.33±1.71	0.43±0.03	0.37±0.01
MeOH (80%)	0.92±0.02	na	35.11±5.77	0.35±0.02	0.30±0.01
Water maceration	0.77±0.08	na	na	0.17±0.01	0.11±0.01
Infusion	0.50±0.08	na	na	0.41±0.01	0.28±0.01

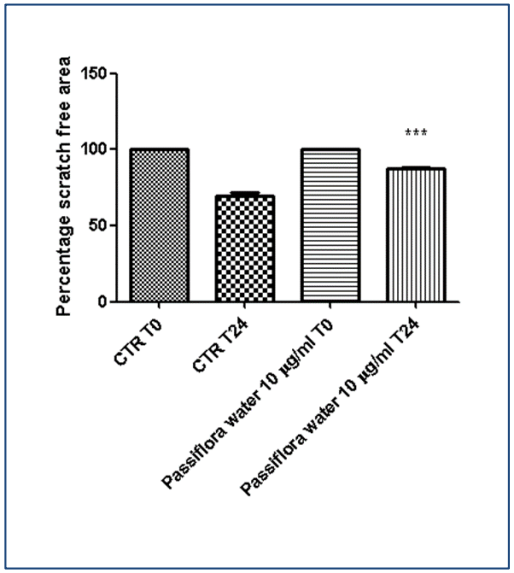
Values are reported as mean ±S.D. EA: Ethyl acetate; GALAE: Galantamine equivalent; KAE: Kojic acid equivalent; ACAE: Acarbose equivalent. na: not active

- Ethyl acetate extract inhibited BChE selectively
- Methanolic extract was observed to be the most potent AChE inhibitor
- Water maceration, methanolic (80%) and infusion extracts showed moderate inhibition against AChE
- Only the ethyl acetate and the two methanolic extracts showed anti-tyrosinase inhibitory potentials
- All tested extracts demonstrated dual inhibition against α-amylase and α-glucosidase

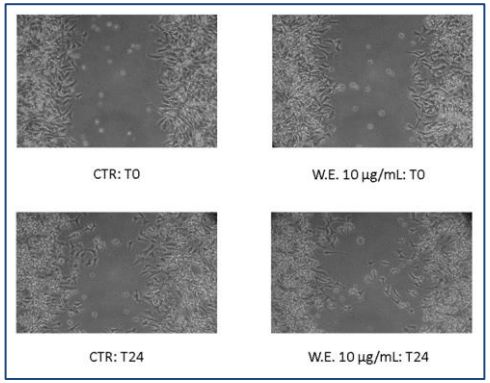
Results and discussion-Biocompatibility



Effects of P. foetida methanol (M.E.) and water (W.E.) extracts on basal and hydrogen peroxide 300 µM (H.P.)-induced hypothalamic HypoE22 cell viability (MTT test). Cell viability was relatively calculated towards the untreated control (CTR) group. Data were analyzed through analysis of variance (ANOVA), followed by post hoc Newman-Keuls test. ANOVA, P<0.001; ***P<0.001 vs. H.P.



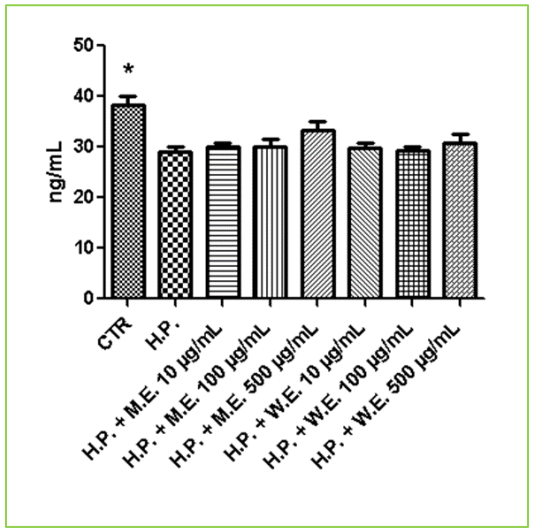
$LC_{50} \leq 5$ mg/mL



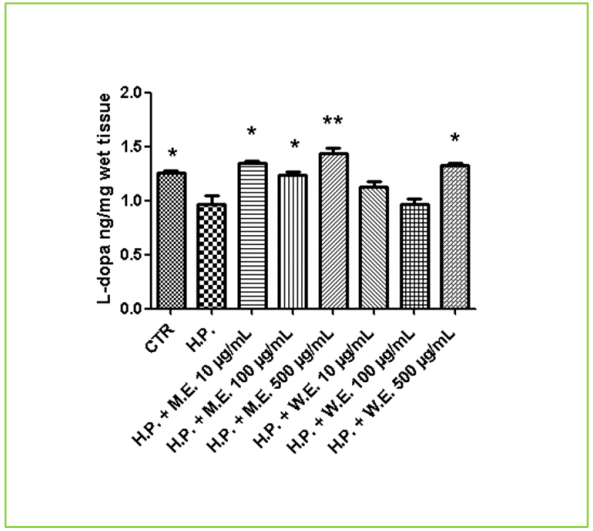
Effects of subtoxic concentration (10 µg/mL) of P. foetida water extract on spontaneous hypothalamic HypoE22 cell migration. Quantification of free cell area (Subfigure A) and representative images (Subfigure B) of wound healing test recorded at different time (T) points:0 and 24 hours following stimulation

- Methanol and water extracts were tolerated by HypoE22 cells in both basal and hydrogen peroxide-induced oxidative stress condition
- The wound healing test showed the water extract-induced decrease in the spontaneous migration showing a minor role exerted by extract as neuroprotective agent

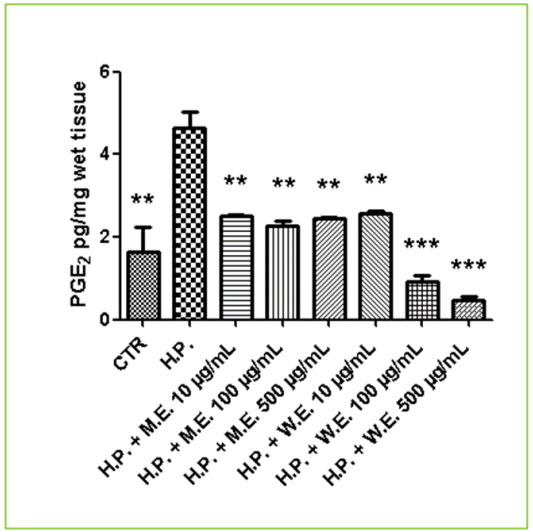
Results and discussion-Neuroprotetctive effects



Effects of P. foetida methanol (M.E.) and water (W.E.) extracts on hydrogen peroxide 300 µM (H.P.)-induced decrease of serotonin (5-HT) release from hypothalamic HypoE22 cell. 5-HT release was quantified through HPLC coupled to coulometric detection and expressed as ng/mL. Data were analyzed through analysis of variance (ANOVA), followed by post hoc Newman-Keuls test. ANOVA, P<0.05; *P<0.05 vs. H.P.



Effects of P. foetida methanol (M.E.) and water (W.E.) extracts on hydrogen peroxide 1 mM (H.P.)-induced decrease of l-dopa release from isolated mouse skin. l-dopa release was quantified through HPLC coupled to coulometric detection and expressed as ng/mg wet tissue. Data were analyzed through analysis of variance (ANOVA), followed by post hoc Newman-Keuls test. ANOVA, P<0.001; *P<0.05, **P<0.01 vs. H.P.



Effects of P. foetida methanol (M.E.) and water (W.E.) extracts on hydrogen peroxide 1 mM (H.P.)-induced decrease of PGE2 release from isolated mouse skin. PGE2 release was quantified through radioimmunoassay and expressed as pg/mg wet tissue. Data were analyzed through analysis of variance (ANOVA), followed by post hoc Newman-Keuls test. ANOVA, P<0.001; **P<0.01 vs. H.P.

- None of the two extracts were able to prevent the hydrogen peroxide-induced decrease of extracellular 5-HT level
- Both extracts were able to prevent L-dopa turnover confirming the anti-tyrosinase activity
- The extracts were effective in blunting hydrogen peroxide-induced level of PGE_2

Results and discussion-Antimicrobial activity

Minimal inhibitory concentrations (MICs) of P foetida methanol and water extracts, and griseofulvin towards selected dermatophytes

Dermatophytes (ID strain) A3:D15	Minimum Inhibitory Concentration (MIC)		
	Methaol Extract (μg mL^{-1}) *	Water Extract (μg mL^{-1}) *	Griseofulvin (μg mL^{-1}) *
Arthroderma crocatum (CCF 5300)	12.4 (7.81–15.625)	39.37(31.25–62.5)	>8
Arthroderma curreyi (CCF 5207)	6.19 (3.9–7.81)	12.4(7.81–15.625)	>8
Arthroderma gypseum (CCF 6261)	157.49 (125–250)	396(250–500)	1.587(1–2)
Arthroderma insingulare (CCF 5417)	12.4 (7.81–15.625)	19.68(15.625–31.25)	>8
Arthroderma quadrifidum (CCF 5792)	78.74 (62.5–125)	314.98(250–500)	>8
Trichophyton mentagrophytes (CCF 4823)	49.6 (31.25–62.5)	99.21(62.5–125)	2.52(2–4)
Trichophyton mentagrophytes (CCF 5930)	157.49 (125–250)	396(250–500)	3.174(2–4)
Trichophyton rubrum (CCF 4933)	39.37 (31.25–62.5)	99.21(62.5–125)	1.26(1–2)
Trichophyton rubrum (CCF 4879)	99.21 (62.5–125)	198,42(125–250)	3.175(2–4)
Trichophyton tonsurans (CCF 4834)	9.84 (7.81–15.625)	9.84(7.81–15.625)	0.198(0.125–0.25)

- MIC values are reported as geometric means of three independent replicates (n = 3); MIC ranges are reported within brackets. MIC values are reported as < [lowest concentration tested]

- Both extracts were able to inhibit fungal growth
- These results add to the aforementioned inhibitory activity against tyrosinase
- The present antimycotic activity could be related, at least partially, to the extract phenol and flavonoid content of *P. foetida*
- Possible phytotherapy use of *P. foetida* as a skin protective agent

Conclusions

- *P. foetida* extracts prepared using different solvents were found to yield varying amount of total phenolic and flavonoid contents. Particularly, ethyl acetate and methanolic (80%) extracts were found to contain the highest phenolic and flavonoid contents respectively. Nonetheless, the infusion and methanolic extracts were both found to be rich in flavonoids. Additionally, extracts of *P. foetida* were revealed to possess notable enzyme inhibitory effects by acting as inhibitors of amylase, glucosidase, tyrosinase, acetyl- and butyryl-cholinesterase, although the extracts differed in their inhibition capacities.
- All *P. foetida* extracts also showed good overall antioxidant potentials by acting as radical scavenging, reducing, and metal chelating mechanisms. The enzyme inhibition and antioxidant effects were also confirmed by pharmacological evaluations. Finally, the extracts were also effective against multiple dermatophytes strains involved in skin inflammation.
- Hence, the results obtained from the current investigation evidently support the therapeutic properties of *P. foetida* as antioxidant, antidiabetic, and anti-hyperpigmentation agents and thus could help to stimulate further consideration to understand their potential influence on human health as potential pharmaceuticals and nutraceuticals

Acknowledgments

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