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HPTLC Fingerprinting of Phenolic Acids and Assessment of Antioxidant Potential of *Pouzolzia bennettiana* Wight a Medicinal Plant from Nilgiri Hills

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Authors' contributions

This work was carried out in collaboration between all authors. Author PNP designed the study, wrote the protocol, performed the analysis and wrote the first draft of the manuscript. Authors GJJ, AR and RA provided assistance and supervised the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To screen the secondary metabolites, evaluate the antioxidant potential and identify phenolic acids of methanolic extract of leaf and stem of *Pouzolzia bennettiana Wight.*
Study Design: The study aimed to determine the antioxidant potential using various assays and to identify the secondary metabolites using HPTLC fingerprinting.
Place and Duration of Study: KMCH college of Pharmacy, between April 2015 and December 2015.
Methodology: The methanolic leaf and stem extract of *Pouzolzia bennettiana Wight* was screened for its phytochemical components, quantitative analysis of phenols and flavonoids, antioxidant activity by DPPH, ABTS, FRAP and TAC assay. Few phenolic acids responsible for antioxidant activity was identified by HPTLC fingerprinting.
Results: The important secondary metabolites such as flavonoids, tannins, terpenoids, saponins

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and sterols were identified in the methanolic leaf and stem extract along with primary metabolites such as carbohydrates and proteins. The total phenol and flavonoid content of leaf extract was found to be 34.65 µg and 33.36 µg/100 µg of extract respectively whereas stem extract possessed 31.74 µg and 12.58 µg/100 µg of extract respectively. The antioxidant potential of the methanol extract of leaf and stem was evaluated and the leaf extract showed significant activities in all antioxidant assays compared to the reference antioxidant whereas the stem possessed less antioxidant activity. The HPTLC fingerprinting of leaf extract revealed the presence of the following phenolic acids orcinol, ferulic acid, benzoic acid and resorcinol. The stem extract identified phloroglucinol, ferulic acid and resorcinol.

Conclusion: These results suggest that *Pouzolzia bennettiana* Wight may act as a potential natural antioxidant offering effective protection from free radicals. The antioxidant effect may be due to the presence of phenolic acids. The phenolic acids identified possess various other applications along with antioxidant activity.

Keywords: Pouzolzia bennettiana; secondary metabolites; antioxidant; HPTLC; phenolic acids.

1. INTRODUCTION

Plants possess several starting material for drug development. Ethnobotany reveals the relationship that exists between people and plants. Many plants are cheaper and more accessible to most people [1]. However loss of biodiversity, over-exploitation and unscientific use of medicinal plants, industrialization, bio piracy, lack of regulation and infrastructure are the major drawbacks to the growth of herbal medicine. Conservation, proper research in traditional knowledge, quality control of herbal medicine and proper documentation is the need of the hour. Health systems around the world are experiencing increased levels of chronic illness and escalating health care costs. Patients and health care providers alike are demanding that health care services be revitalized, with a stronger emphasis on individualized, person-centred care [2]. This includes expanding access to traditional products, practices and practitioners.

Phytochemicals are compounds found in plants that are not required for normal functioning of the body, but have a beneficial effect on health or play an active role in amelioration of diseases [3]. Diseases in the present day are due to shift in the balance of the pro-oxidant and antioxidant homeostatic phenomenon in the body. Excessive oxidative stress of the modern life leads to pro-oxidant conditions either due to increase in generation of free radicals or poor scavenging or quenching in the body caused by lack of natural and dietary antioxidants. Oxidative damage is a critical etiological factor implicated in several chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis, neurodegenerative diseases and ageing process [4].

Natural antioxidants from plant sources are a group of compounds with different chemical structures. These antioxidants neutralise the free radicals by scavenging them. Medicinal plants almost contain polyphenolic phytochemicals which act as powerful antioxidants by adsorbing or neutralizing the free radicals, quenching singlet and triplet oxygen or decomposing peroxides [5]. Therefore screening of plants on the basis of their antioxidant activity is a challenge for the scientists. The bio active compounds are of very much interest for the scientific advancement through natural antioxidants.

HPTLC fingerprinting technique is widely used in recent years to identify, authenticate and estimate the active constituents with reasonable accuracy in a shorter time. It has become the most potent tool for quality control of herbal medicines because of its simplicity and reliability [6].

Pouzolzia bennettiana is a rare valuable medicinal plant found in hills of India and Sri Lanka. It is a Shrubby plant belonging to the family Urticaceae. It is commonly called as chandermuli, naralikola, oyik and serathandan. Ethnomedicinal information revealed that it is used as antiseptic, to treat cuts, for bone fracture and dislocation and heal skin burn with inflammation. In chakma community *Pouzolzia bennettiana* is eaten as vegetable by expecting mothers to increase secretion of milk [7].

In spite of the numerous medicinal uses attributed to this plant, there is no pharmacognostical report for methanolic hot extract of *Pouzolzia bennettiana*. Natural antioxidants can exert their effect through a

variety of different mechanisms, it is important to prove their antioxidative activity. The purpose of the present study is to assess phytochemicals, the total flavonoids content, the total phenolic content, to screen the extract for their antioxidant potential using *in vitro* assays and to identify phenolic acids using HPTLC fingerprinting.

2. MATERIALS AND METHODS

2.1 Plant Collection and Authentication

The whole plant material of *Pouzolzia bennettiana* was procured from Kotagiri, The Nilgiris, Tamil Nadu, India and the plant was taxonomically authenticated by the Scientist in Botanical Survey of India, TNAU campus Coimbatore. The voucher number BSI/SRC/ 5/23/2012-13/Tech./793.

The collected plants were washed thoroughly with running tap water to remove the unwanted debris. The leaves and the stem were separated and shade dried for about two weeks and then powdered using pulverizer. The powder obtained was stored in air tight containers and left at room temperature. The powder was used for preparation of extract.

2.2 Solvent Extraction

The leaf and stem powder was subjected to successive solvent extraction in Soxhlet apparatus using petroleum ether and methanol. 200 g of coarsely powdered plant material was soaked in 2 L petroleum ether for 3 days. The filtrate was removed and concentrated in a rotary evaporator under reduced pressure at 40-50℃. The marc was re-extracted twice with petroleum ether at three day intervals. All the extracts were pooled and the colour, consistency and percentage yield of the extract were noted. The petroleum ether extracted marc was then sequentially extracted with methanol using the same procedure as above. The solvent was removed under the vacuum at temperature below 50℃ and the extracts were freeze-dried. All the extracts were stored in vacuum dessicator for further studies.

2.3 Qualitative Phytochemical Screening

Phytochemical screening was performed using standard methods described by Sofowora, Trease and Evans and Harbone [1,8,9].

2.4 Quantitative Phytochemical Determination

2.4.1 Determination of total flavonoids content

Total flavonoid content was determined following a method by Park et al. [10]. Quercetin was used as standard. Concentrations of 5 µg/mL, 10 µg/mL, 25 µg/mL, 50 µg/mL, 75 µg/mL and 100 µg/mL of quercetin, leaf and stem extracts were prepared in distilled water. 0.3 mL of extracts, 3.4 mL of 30% methanol, 0.15 mL of NaNO2 (0.5 M) and 0.15 mL of AlCl3.6H2O (0.3 M) were mixed. After 5 min, 1 mL of NaOH (1 M) was added. The solution was mixed well and the absorbance was measured against the reagent blank at 506 nm spectrophotometrically. All determinations were performed in triplicates. The total flavonoids were expressed as milligrams of quercetin equivalents per gram of dry weight.

2.4.2 Determination of total phenolic content

Total phenolic content was determined according to the Folin and Ciocalteau's method. Gallic acid was used as a standard. Concentrations of 100 µg/mL, 200 µg/mL, 400 µg/mL, 600 µg/mL, 800 µg/mL and 1000 µg/mL of gallic acid, leaf and stem extracts were prepared in distilled water. 0.5 mL of each sample was mixed with 2.5 mL of a ten-fold diluted Folin-Ciocalteau's reagent and 2 mL of 7.5% sodium carbonate. The mixture was then allowed to stand for 2 hours at room temperature before the absorbance was read at 760 nm spectrophotometrically. All determinations were performed in triplicates. The total phenolic content was expressed as gallic acid equivalent per gram of dry weight (mg GAE/g dry weight), which is a common reference compound [11].

2.5 *In vitro* Antioxidant Studies

The leaf and stem extract was dissolved in 95% methanol to make a concentration of 1 mg/mL and then diluted to prepare the series concentrations for antioxidant assays. DPPH, ABTS, FRAP and Total antioxidant capacity assays were performed to evaluate the antioxidant capacity of *Pouzolzia bennettiana.* Reference chemicals were used for comparison in all assays.

2.5.1 DPPH photometric assay

Antioxidant activity was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) stable radical

method in MeOH solution [12]. Sample stock solutions (1.0 mg/mL) were diluted to final concentrations of 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL and 100 µg/mL in methanol. One mL of 0.3 mm DPPH methanolic solution was added to 2.5 mL of sample solutions of different concentrations and allowed to react at room temperature. After 30 minutes the absorbance values were measured at 518 nm and converted into percentage of antioxidant activity (AA) using the following formula:

$$AA\ \% = 100 - \{ [(ABS_{SAMPLE} - ABS_{BLANK}) \times 100] / ABS_{CONTROL}\}$$

Methanol (1.0 mL) plus extract solution (2.5 mL) was used for blank. 1mL of 0.3 mm DPPH solution and 2.5 mL of methanol was used as negative control. The positive controls were those using the standard solutions. Average percent of antioxidant activity from three separate tests were calculated [13]. The results are also presented as radical scavenger capacity, expressed as IC_{50}, which is the amount of antioxidants necessary to decrease the initial DPPH. Solution of ascorbic acid were prepared in similar concentrations and used as antioxidant standards.

2.5.2 2,2´-Azinobis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS+) radical scavenging activity

ABTS is converted to its radical cation by addition of sodium persulfate. Ascorbic acid in ethanol was used as standard. ABTS.+ radical cation was produced by the reaction of 7 mM ABTS solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature before use. The ABTS.+ solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. 2 mL of diluted ABTS.+ solution was added to 25 µL of sample and after five minutes absorbance was read at 734 nm [14]. Ascorbic acid was used as standard. Percentage inhibition was calculated using the formula.

Percentage inhibition= [1− (absorbance of test/absorbance of control)]×100

2.5.3 Ferric reducing ability of plasma (FRAP) expressed as a function of time

FRAP agent was prepared by mixing 25 mL of acetate buffer (500 mM/L) with 2.5 mL of tripyridyltriazine (TPTZ) (10 mM/L) and 2.5 mL of ferric chloride (20 mM/L) solutions. Gallic acid was used as standard. The reaction mixture of 300 µL of freshly prepared FRAP reagent warmed to 37℃ for ten minutes and then added 10 µL of leaf and stem extract along with 30 µL of distilled water. Absorbance of this solution was taken at 593 nm just after 4 min from the time of addition of FRAP reagent. An increase in absorbance indicated enhanced reducing potential of plasma [15].

2.5.4 Phosphomolybdate assay (Total antioxidant capacity)

The total antioxidant capacity (TAC) of extracts and standard were evaluated following the method of Prieto et al. [16]. Quercetin was used as standard. An aliquot of 0.1 mL of sample solutions (1 mg/mL) were combined with 1 mL of reagent solution (600 mM sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95℃ for 90 min. After the samples were cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. A typical blank solution contained 1 mL of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under the same conditions. The antioxidant capacity was expressed as the number of equivalents of quercetin (µg/g of extract).

The antioxidant capacity was estimated using following formula:

$$\text{Antioxidant effect (\%)} = \frac{(\text{control absorbance - sample absorbance})}{\text{Control absorbance}} \times 100$$

2.6 HPTLC Analysis for Phenolic Acids

HPTLC analysis were carried out for the leaf and stem extracts of *Pouzolzia bennettiana* [17]. All the solvents used for HPTLC analysis were obtained from MERCK. 100 mg leaf and stem extract was dissolved in 1 mL of methanol and the solution was centrifuged at 3000 rpm for 5 min and used for HPTLC analysis as test solution. 5 µl of test solution and 5 µl of standard solutions such as vanillin, salicylic acid, benzoic acid, phloroglucinol, cinnamic acid, ferulic acid, resorcinol and orcinol were loaded as 5 mm band length in the 10 x 10 Silica gel 60F254 with 250 µm thickness (E-Merck, Darmstadt, Germany)

TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The samples loaded plate was kept in TLC twin trough developing chamber (after saturation with Solvent vapour) with respective mobile phase Ethyl acetate: benzene (9:11) [18] up to 80 mm. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at UV 254 nm and UV 366 nm. The plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at 500 nm. The developed plate was sprayed with anisaldehyde sulphuric acid and dried at 100℃ in hot air oven for 3 min.

3. RESULTS AND DISCUSSION

Plants constitute an important source of active natural products. Nutrition and health care are strongly interconnected and many plants have been consumed both as food and for medicinal purposes during recent years. The consumption of non-cultivated plants plays an important role in the diet of traditional society, but very few ethnopharmacological and phytopharmacological studies have dealt exhaustively with the potential health benefits of such diets. *P. bennettiana* is considered as a nutritious traditional medicinal plant used for medicinal purpose and to increase lactation in breast feeding mothers [7]. The antioxidant potential and phenolic acids is responsible for antioxidant potential of *P. bennettiana* (Urticaceae) is unexplored to some extent. Hence in this study the antioxidant capacity of *P. bennettiana* was evaluated using antioxidant assays and few phenolic acids were identified by HPTLC fingerprinting.

3.1 Screening of Secondary Metabolites

The results of qualitative phytochemical screening of petroleum ether and methanol extract of stem and leaf of *Pouzolzia bennettiana* revealed the presence of alkaloid, carbohydrate, protein, tannin, terpenoid, saponin, sterol and flavonoids in methanol extract of leaf and stem and presence of carbohydrate, protein, terpenoid and sterols in petroleum ether extracts.

3.2 Quantitative Analysis

3.2.1 Total phenol content and flavonoids content

The preliminary phytochemical analysis showed the presence of phenols and flavonoids in both leaf and stem which are considered as potent antioxidants. Phenols and Flavonoids are the largest group of plant secondary metabolites that possess multiple biological actions or effects [19,20]. The total phenol and flavonoids was determined by UV spectrophotometric method. Table 1 summarises the total phenolic content and total flavonoid content of the methanolic extract of leaf and stem.

The leaf exhibited the highest total phenolic content and total flavonoids content compared to stem. The marked antioxidant ability of plant extract is due to its high phenolic or polyphenolic content.

3.3 Assessment of Antioxidant Potential

The antioxidant activity of phenols is mainly due to their redox properties which make them act as reducing as agents, hydrogen donors, and singlet oxygen quenchers. They also may have a metallic chelating potential [21,22]. Flavonoids are highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various free radicals [23] implicated in several diseases.

The methanol extract of leaf and stem were compared with standard markers to evaluate their antioxidant capacity using DPPH photometric assay, ABTS+ radical scavenging activity, Ferric reducing ability of plasma (FRAP) and Phosphomolybdate assay (total antioxidant capacity). Table 2 indicates the IC_{50} values of standard, leaf and stem.

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay is the most commonly used method for evaluation. The DPPH radical is considered to be a model of lipophilic radical and it is a free radical that accepts electron or hydrogen radical to become stable diamagnetic molecule. DPPH was reduced to pale yellow due

Table 1. The total phenolic content and total flavonoid content of the methanolic extract of leaf and stem

Sample	Total phenol content (µg gallic acid /100 µg of extract)	Total flavonoids content (µg quercetin/ 100 µg of extract)
Leaf	34.65 µg	33.36 µg
Stem	31.74 µg	12.58 µg

Table 2. Antioxidant potential of standard, leaf extract and stem extract of *P. bennettiana*

Assay	IC_{50} (µg/mL)		
	Standard	Leaf	Stem
DPPH (Ascorbic acid)	66.26	71.29	99.47
ABTS (Ascorbic acid)	54.99	79.05	107.2
FRAP (Gallic acid)	27.40	69.28	85.76
TAC (Quercetin)	10.96	18.64	56.76

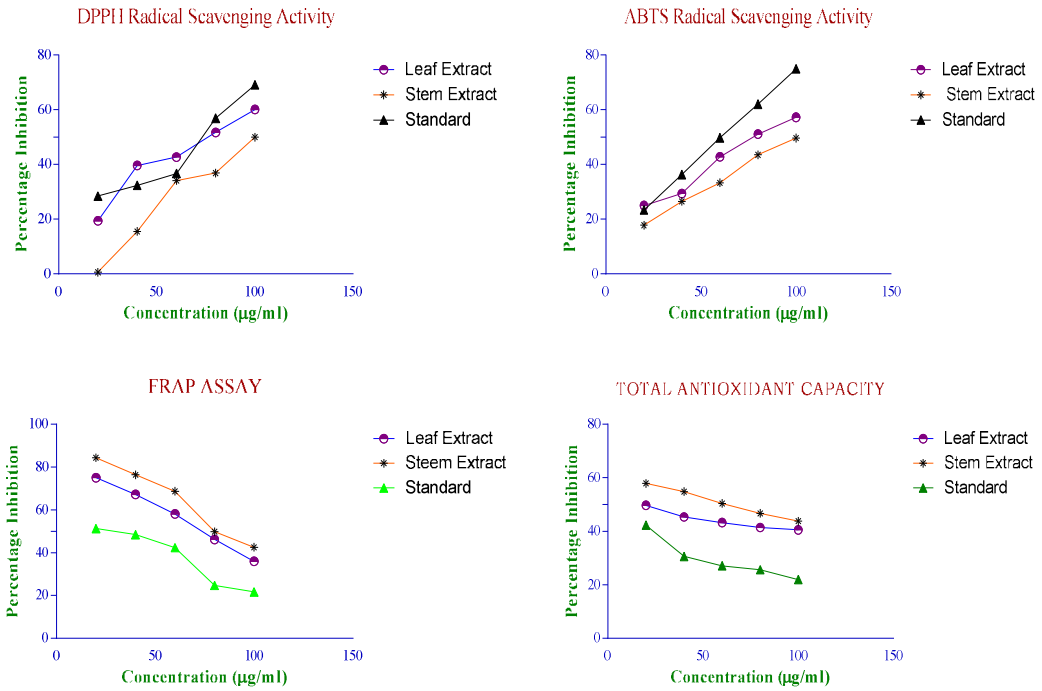

Fig. 1. Antioxidant potential of standard, leaf extract *and* stem extract of *P. bennettiana*

to abstraction of hydrogen atom from antioxidant compound and read at 517 nm [24]. IC_{50} is the amount of antioxidant present in the sample necessary to decrease the initial DPPH concentration by 50%. Lower IC_{50} value indicates higher antioxidant activity. The present study indicated that the leaf extract possess good scavenging activity while stem extract has moderate activity compared to standard ascorbic acid. ABTS radical cation was not seen in the body. The antioxidant compounds in the extract reduces ABTS+ to ABTS and read at 734 nm [25]. The leaf and stem extracts were evaluated and observed to posses moderate TEAC activity when compared to standard ascorbic acid. FRAP assay measures the reductants [26], that possess the ability to reduce ferric ion measured at 593 nm. The leaf extract has moderate activity where as stem has poor ability to reduce ferric ion. The quantitative phosphomolybdenum method assessed the total antioxidant capacity based on the reduction of Mo (VI) to Mo (V). Earlier studies indicated that plants belonging to Urticaceae family such as *Pouzolzia zeylanica* [27], *Urtica dioca* [28], *Pilea microphylla* [29] and *Fluery aaestuans L* [30] posses good antioxidant activity and can be considered as natural antioxidant. The IC_{50} value of TAC assay indicated that the leaf can be considered as good or potent antioxidant but stem possess very low antioxidant potential when compared to leaf and standard.

3.4 HPTLC Fingerprinting of Phenolic Acids

The methanolic extract of leaf and stem showed the presence of many peaks with different R_f values. 10 peaks were obtained in leaf ranging from 0.16 to 0.85 and 7 peaks in stem ranging

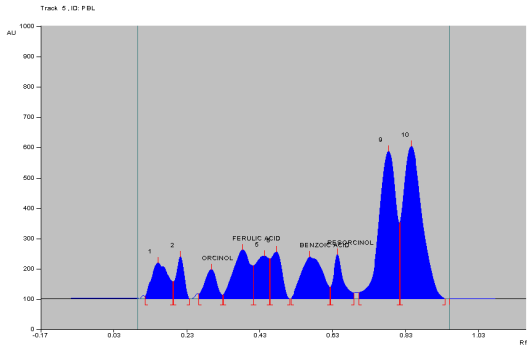

Fig. 2a. HPTLC chromatogram of *P. bennettiana* methanolic leaf extract peak densitogram display at 500 nm

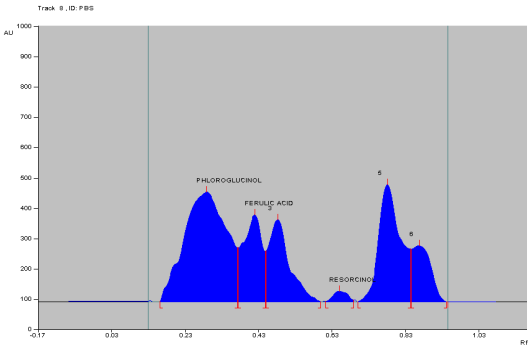

Fig. 2b. HPTLC chromatogram of *P. bennettiana* methanolic stem extract peak densitogram display at 500 nm

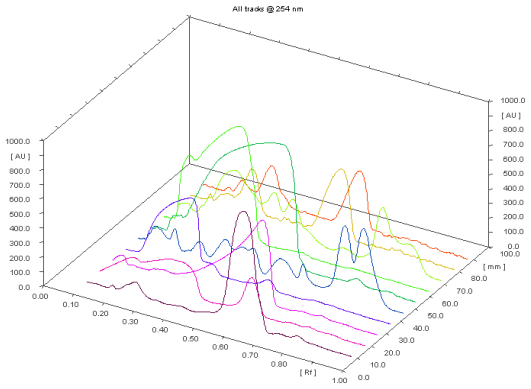

Fig. 3. 3D display of HPTLC chromatogram of *P. bennettiana* leaf, stem and phenolic standard

from 0.13 to 0.87. Leaf extract showed the presence of Orcinol, Ferulic acid, Benzoic acid and Resorcinol whereas stem possessed Phloroglucinol, Ferulic acid and Resorcinol.

Plant materials rich in phenols are increasingly being used in the food industry because they retard oxidative degradation of lipids and improve the quality and nutritional value of food [31]. Chromatographic finger printing of phytoconstituents can be used to assess the quality consistency and stability of herbal extract [32]. It involves comparison between standard and sample. The markers usage ensures the components present in the plant. Phenolic compound ferulic acid was identified in *Urtica dioca* [28]. The phenolic acids orcinol, ferulic acid, benzoic acid, resorcinol and phloroglucinol were identified in the extracts possess valuable medicinal properties such as antioxidant, antiallergic, hepatoprotective, anticarcinogenic, anti-inflammatory, antimicrobial, spasmolytics, anthelminthic, to treat acne, eczema, psoriasis, seborrhea and other skin disorders [33-36]. The blue bands at 366 nm and violet and brown bands similar to standard after spraying anisaldehyde sulphuric acid indicated the presence of phenolic acids.

4. CONCLUSION

The phytochemical and *in vitro* antioxidant studies proves that the methanolic extract of leaf and stem of the hill plant *Pouzolzia bennettiana* is a potent antioxidant. The HPTLC fingerprinting added more information about the plant to be considered as important dietary sources of phenolic antioxidants. The study confirms interface of traditional therapy and scientific proof. This useful wild plant which is consumed as vegetable requires further investigation on the active components and therapeutic efficacy.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sofowora A. Medicinal plants and traditional medicine in Africa. Ibadan, Nigeria: Spectrum Books Ltd; 1993.
2. Roberti SP, Mauro A, Guadagni P. Widening the paradigm in medicine and

health: Personcentred medicine as the common ground of traditional, complementary, alternative and non-conventional medicine. In: Health care overview: New perspectives, advances in predictive, preventive and personalised medicine. Dordrecht, Springer. 2012;1:335 –353.

3. Pamplona RD. Encyclopedia of medicinal plants. Saeliz, Spain; 1998.
4. Dringen R. Glutathione metabolism and oxidative stress in neurodegeneration. Eur J Biochem. 2000;267(16):4903.
5. Kumawat BK, Gupta M, Tarachand SY. Free radical scavenging effect of various extracts of leaves of *Balanite aegyptica* (L.) Delile by DPPH method. Asian J of Plant Science and Research. 2012;2(3):323-329.
6. Ram M, Abdin MZ, Khan MA, Jha P. HPTLC fingerprint analysis: A quality control of authentication of herbal phytochemicals. Springer. 2011;105.
7. Sarmah R, Adhikari D, Majumder M, Arunachalam A. Traditional medicobotany of chakma community residing in the Northwestern Periphery of Namdapha National Park in Arunachal Pradesh. Indian Journal of Traditional knowledge. 2008; 7(4):587-593.
8. Trease GE, Evans WC. Pharmacognosy. 11th ed. London: Brailliar Tiridel and Macmillian Publishers; 1989.
9. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. 3rd ed. London: Chapman and Hall Ltd; 1973.
10. Park YS, Jung ST, Kang SG, Heo BK, Arancibia Avila P, Toledo F, Drzewiecki J, Namiesnik J, Gorinstein S. Antioxidants and proteins in ethylene-treated kiwifruits. Food Chem. 2008;107:640–648.
11. Folin O, Ciocalteau V. On tyrosine and tryptophane determination in proteins. Journal of Biol. Chem. 1927;27:627-650.
12. Brandwilliams W, Cuvelier ME, Berset C. Use of a free-radical method to evaluate antioxidant activity. Food Sci Technol Lebensmittel-Wissenschaft & Technologie. 1995;28:25-30.
13. Mensor LL, Menezes FS, Leitao GG, Reis AS, dos Santos TC, Coube CS, Leitao SG. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res. 2001; 15:127-130.
14. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 1999;26(9-10):1231-1237.
15. Iris F, Benzie F, Strain JJ. The Ferric Reducing Ability of Plasma (FRAP) a measure of ''Antioxidant Power'': The FRAP assay. Analytical Biochemistry. 1996;239:70-76.
16. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem. 1999;269(2): 337-341.
17. Misra H, Mehta BK, Jain DC. Optimization of extraction conditions and HPTLC-UV method for determination of quinine in different extracts of *Cinchona* species bark. Rec Nat Prod. 2008;2(4):107-115.
18. Wagner H, Bladt S. Plant drug analysis. 2nd ed. Springer Verlag Berlin. 1996;195-244.
19. Soobrattee MA, Neergheen VS, Luximon-Ramma A, Aruoma OI, Bahorun T. Phenolics as potential antioxidant therapeutic agents: Mechanism and action. Mutation Research. 2009;579:200-213.
20. Osawa T. Novel natural antioxidants for utilization in food and biological systems. In: Uritani I, Garcia VV, Mendoza EM, (Eds). Post harvest biochemistry of plant food-materials in the tropics. Japan Scientific Societies Press. 1994;241-251.
21. Olajire AA, Azeez L. Total antioxidant activity, phenolic, flavonoid and ascorbic acid content of Nigerian vegetables. African Journal of Food Science and Technology. 2011;2(2):22-29.
22. Rice Evans CA, Miller MJ, Bolwell PG, Bramley PM, Pridham JB. The relative antioxidant activities of plant derived polyphenolic flavonoids. Free Radical Research. 1995;22:375–383.
23. Bravo L. Polyphenols: Chemistry, dietary sources, metabolism and nutritional significance. Nutr Reviews. 1998;56:317–333.
24. Lachumy SJT, Sasidharan S, Sumathy V, Zuraini Z. Pharmacological activity, phytochemical analysis and toxicity of methanol extract of *Etlingera elatior* (torch ginger) flowers. Asian Pac J Trop Med. 2010;3(10):769-774.
25. Miller NJ, Rice Evans CA. Factors influencing the antioxidant activity

determined by the ABTS•1 radical cation assay. Free Radic. Res. 1997;26:195–199.
26. Duh PD, Tu YY, Yen GC. Antioxidant activity of the aqueous extract of harn jyur (*Chrysanthemum morifolium* Ramat). Lebensmittel-Wissenschaft and Technologie. 1999;32:269-277.
27. Li P, Huo L, Su W, Lu R, Deng C, Liu L, Deng Y, Guo N, Lu C, He C. Free radical-scavenging capacity, antioxidant activity and phenolic content of *Pouzolzia zeylanica*. J. Serb. Chem. Soc. 2011; 76(5):709–717.
28. Vertika K, Pradeep K, Shikhar V, Abhishek G, Sharad S, Ajay KSR. Pharmacognostic evaluation and antioxidant activity of *Urtica dioica* L. Chinese Medicine. 2012;3:128-135.
29. Amir MC, Darah I, Shaida FS. Antioxidant, antimicrobial activity and toxicity test of *Pilea microphylla*. International Journal of Microbiology. 2010;1-6.
30. Akinlami OO, Lajide L, Owolabi BJ, Osho IB. Phytochemical constituent and antioxidant potential of the leaf extract of *Fluery aaestuans* L (Urticaceae). Global Advanced Research Journal of Medicine and Medical Science. 2014;3(10):331-334.
31. Kähkönen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M. Antioxidant activity of plant extracts containing phenolic compounds. J Agri Food Chem. 1999;47:3954–3962.
32. Mariswamy Y, Gnaraj WE, Antonisamy JM. Chromatographic fingerprint analysis on flavonoids constituents of the medicinally important plant *Aerva lanata* L. by HPTLC technique. Asian Pac J Trop Biomed. 2012;8-12.
33. Graf E. Antioxidant potential of ferulic acid. Free Radic. Biol. Med. 1992;3:435–513.
34. Boer J, Jemec GB. Resorcinol peels as a possible self-treatment of painful nodules in hidradenitis suppurativa. Clin Exp Dermatol. 2010;35(1):36–40.
35. Charles OW, Ole G, John HB. Wilson and Gisvold's textbook of organic medicinal and pharmaceutical. Lippincott Williams & Wilkns. 2004;234.
36. Inder PS, Jasmeen S, Parikshit B, William JF. Phloroglucinol compounds of therapeutic interest: Global patent and technology status. Expert Opinion on Thherapgeutic Patents. 2009;847-866.

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