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Research Article

Evaluation and Screening of Natural Antioxidant Biomolecules from Flavonoid Rich Fraction of *Premna corymbosa* Rottl

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ABSTRACT

Objective: To evaluate the screening of natural antioxidant molecules and radical scavenging activities of flavonoid rich fractions from the leaves of *Premna corymbosa*. Methods: The total phenolic contents were estimated by Folin–Ciocalteu method adopted by Djeridane et al and total flavonoid content was measured according to Quettier-Deleu et al method. Antioxidant activities of flavonoid rich fraction of *P. corymbosa* was tested on the basis of ABTS (2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical, Super oxide radical, Nitric oxide radical scavenging activity, metal chelating and reducing power activity. Results: The total phenolic and flavonoid content in flavonoid rich fraction of *P. corymbosa* was 36.8±13.00 mg GAE/g and 78.2±1.23µg RE/g respectively. The flavonoid rich fraction of the leaves of *P. corymbosa* showed significant antioxidant activity. The reducing power of the flavonoid rich fraction was comparable with ascorbic acid and found to be dose dependant. EC_{50} values of flavonoid rich fraction of the leaves of *P. corymbosa* were 13.78, 19.39, 12.55 and 14.44 µg/ml in ABTS, superoxide radicals, Nitric oxide radical scavenging and Metal chelating activities respectively. TLC bioautography analysis revealed the presence of flavonoid compound. GC–MS analysis of the flavonoid rich fraction showed the identification of 14 major compounds such as, Catechol, 1-Hexadecanol, Phenol 2-6 dimethoxy, 1-Decanol,2-hexyl, Benzaldehyde and 2 hydroxy-3-methoxyl. Conclusion: This research work has concluded that the flavonoid rich fraction of *P. corymbosa* possess antioxidant activity and may contribute to the wellbeing of individuals who consume them.

Key Words: *Premna corymbosa,* antioxidant, flavonoid rich fraction, antioxidant molecules.

INTRODUCTION

India is a repository of rich medicinal herbs which have been traditionally used. Medicinal plants played an important role in the treatment of diseases and health disorders for thousands of years and are still important in traditional systems of medicine around the world[1]. The world Health Organization has also estimated conservatively that 60-90% of the population of non-industrialized countries rely, either totally or partially on medicinal plants to meet their health care needs[2]. Tribals depend on the medicinal plants for their day-to-day life starting from food to health care[3]. The ethno botanical reports provide the information on several medicinal plants[4]. In Siddha system of medicine, it is cited as "Food as Medicine". But now the concept is shifted to "Medicine as Food "due to the fast food culture by the modern societies[5]. Even with the discovery of many novel drugs that can cure the disorders, the affordability, especially for those in developing countries is the major limitation. For the past two decades, humans were in search of effective drugs that will combat deadly diseases without any side effects[6].

Non-communicable diseases are increasingly becoming the major threats to the health care systems in the world. It is currently hypothesized that many diseases are due to oxidative stress that results from an imbalance between the formation and detoxification of pro-oxidants. Oxidative stress is initiated by Reactive Oxygen Species (ROS) which are produced as a by-product of electron transport in Mitochondria[7]. ROS is also additionally produced in cells as a response to several factors, including oxidative and thermal stress, ultraviolet, chemical agents and ionizing radiation. Reactive nitrogen and oxygen species like nitric oxide, singlet oxygen, hydrogen peroxide, superoxide, hydroxyl and peroxyl free radicals are widely considered to be the critical part of this epidemiological transition[8]. This is because of the capacity of these reactive species when accumulated within body in excess amount cause oxidative stress leading to DNA damage, cell functions inhibition, lipid and protein peroxidation and disturbance of glutathione levels[9] thereby cause chronic inflammation, cancer, atherogenesis and vascular diseases, pulmonary and age related pathology and diseases[10-12].

Nowadays, there is a noticeable interest in antioxidants, especially in those which can prevent the presumed deterious effects of free radicals in the human body, and to prevent the deterioration of fats and other constituents of food stuffs[13]. In both the cases, there is a preference for antioxidants from natural rather than synthetic sources[14]. At present most of the antioxidants are manufactured synthetically. The main disadvantage with synthetic antioxidants is the side effects in vivo[15]. Previous studied

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reported that Butylated hydroxyanisole (BHA) and Butylated hydroxytoluene (BHT) accumulate in the body and result in liver damage and carcinogenisis[16]. Therefore; strict governmental rules regarding the safety of food have led to search for safer alternatives as food preservatives. Hence, an alternative is the consumption of natural antioxidants from various food supplements and traditional medicines[17] and there is increased interest among the Phytotherapy researchers to use medicinal plants with antioxidant activity for protection against oxidative stress. Hence the aim of the present study was to scientifically evaluate the screening of natural antioxidant molecules and antioxidant properties of *P. corymbosa* using various invitro models.

MATERIALS AND METHODS

Plant collection

The medicinal plant used in the present study was *Premna corymbosa* (Verbenaceae). Leaves were identified and authenticated by Dr. S. Sankaranarayanan M. Sc., Ph. D, Assit. Professor, Department of Botany, Government Arts College, Tiruvanamalai, Tamilnadu, India.

Extraction of Flavonoid rich fraction

The leaves of *P. corymbosa* was collected and dried in hot air oven at (40°C) for 1 week, after which it was ground to uniform powder with house hold mixer grinder. The methanol extracts were prepared by soaking 100 g each of the dry powdered plant materials in 250 ml of methanol at room temperature for 48 h. The extracts were filtered first through a Whatmann filter paper No. 42 (125mm) and then centrifuged at 5000 rpm for 10 min (Remi-R-8C, India). The clear solution was collected and concentrated using a rotary evaporator with the water bath set at 40°C and condensed solution was partitioned with petroleum ether followed by chloroform and finally n-butonal. The n-butonal extract contained rich flavonoid and subjected to further study.

Determination of total Phenolic and flavonoid content

The total amount of phenolic content in flavonoid rich fraction of *P. corymbosa* was determined by Folin-Ciocalteu method Djeridane et al.[18] 0.2 ml of extract was taken in a test tube. To this 0.5 ml distilled water and 0.5 ml Folin-Ciocalteu reagent was added and the tubes were shaken thoroughly. After 1 min. 0.8 ml of sodium carbonate solution (7.5%) was added and the mixture was allowed to stand for 30 min. with intermittent shaking. The absorbance was measured at 760 nm using Deep Vision 1371 spectrophotometer. The concentration of the total phenolics was estimated as mg of gallic acid equivalent by using an equation obtained from gallic acid calibration curve. The quantification of phenolic compounds in all the fractions was carried out in triplicate and the results were averaged. The amount of total flavonoids in the extracts was measured according to Quettier-Deleu et al. [19]. This method is based on the formation of a complex flavonoid-aluminium, with the absorbance maximum at 430nm. Rutin was used to make a calibration curve. To 1ml of flavonoid rich fraction, added 1ml of 2% $AlCl_3$ and it was incubated at room temperature for 15 min. Then absorbance was measured at 430 nm using Deep Vision 1371 spectrophotometer.

Reducing power

The reducing power of flavonoid rich fraction of *P. corymbosa* was determined by the method of Oyaizu.[21] Different concentrations of flavonoid rich fraction (5-20 µg/ml) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50 ^{0}C for 20 min. Aliquots (2.5 ml) of trichloroacetic acid (10%) were added to the mixture, which was then centrifuged for 10 min at 1036Xg (Remi India). The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm in a spectrophotometer. Increased absorbance of the reaction mixture indicates increased reducing power.

ABTS (2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical scavenging assay

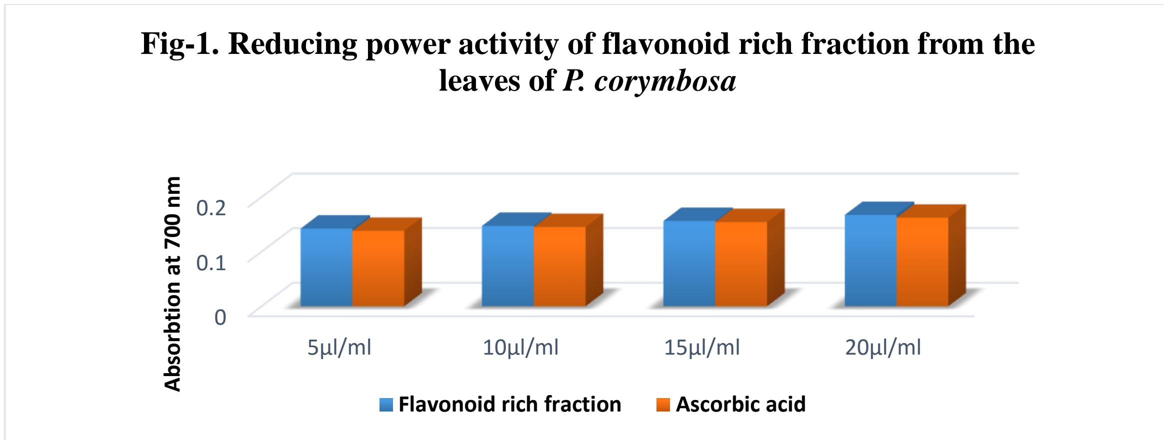
The antioxidant activity of flavonoid rich fraction of *P. corymbosa* was measured by ABTS method as described by Re et al.[20]ABTS radical was freshly prepared by adding 5 ml of 4.9 mM potassium persulfate solution to 5 ml of 14 mM ABTS solution and kept for 16 h in dark. This solution was diluted with distilled water to yield an absorbance of 0.70 at 734 nm and the same was used for the antioxidant assay. The final reaction mixture of standard group was made up to 1 ml with 950 µl of ABTS solution and 50 µl of Ascorbic acid. Similarly, in the test group, 1 ml reaction mixture comprised 950 µl of ABTS solution and 50 µl of different concentration flavonoid rich fraction solution. The reaction mixture was vortexed for 10 s and after 6 min, absorbance was recorded at 734 nm against distilled water by using a Deep Vision (1371) UV–Vis Spectrophotometer and compared with the control ABTS solution. The measurement was performed in triplicate and the percentage decrease of the absorbance at 734nm was calculated by the following formula. The EC_{50} values for the flavonoid rich fraction was calculated and compared with the standard reference compound ascorbic acid. EC_{50} values denote the concentration of sample required to scavenge 50% ABTS free radicals.

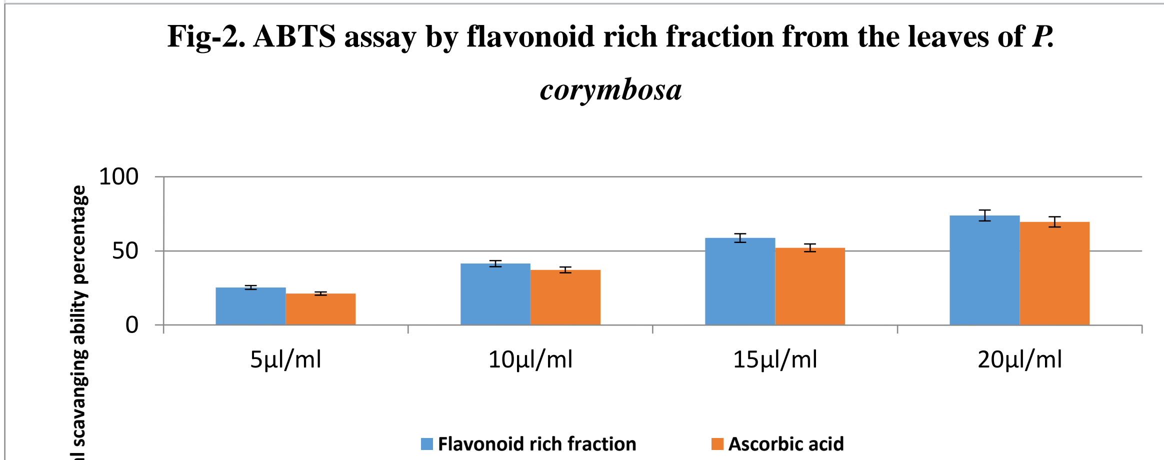
ABTS Scavenging Effect (%) = $[(A_0 - A_1/A_0) \times 100]$

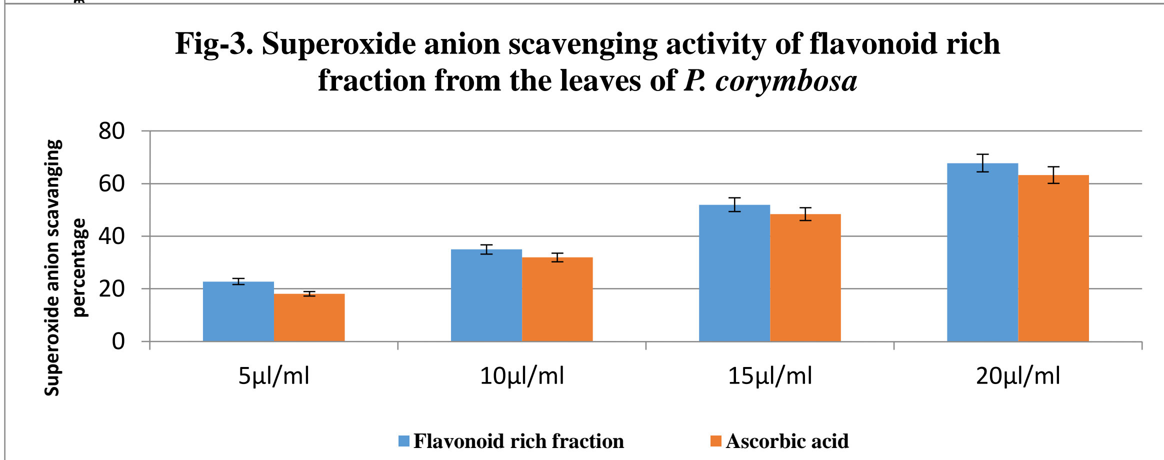
Where A_0 is the absorbance of the control reaction and A_1 is the absorbance of flavonoid rich fraction or standard Ascorbic acid

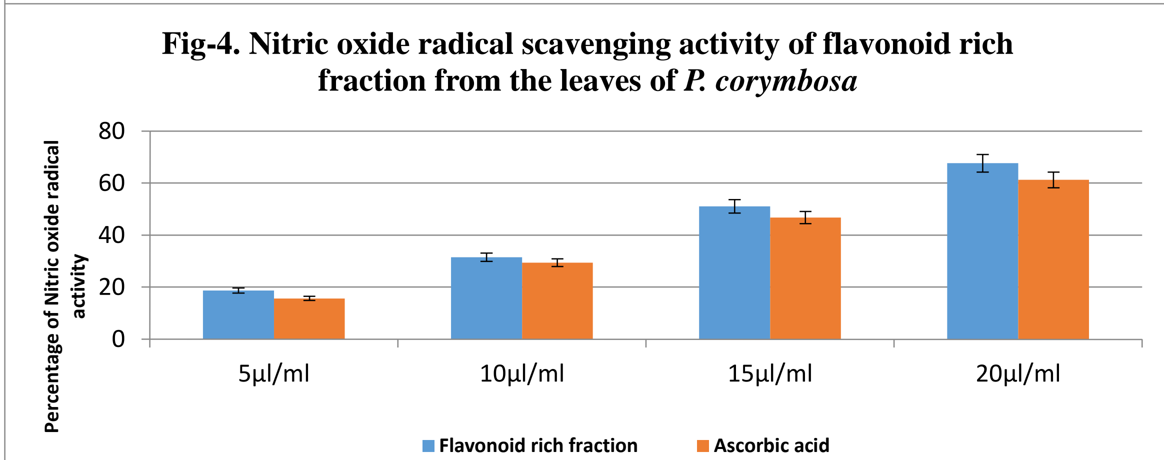
Superoxide radical scavenging assay

The Superoxide scavenging ability of flavonoid rich fraction was assayed following the method of Tripathi and Pandey[22]; Tripathi and Sharma[23]. and the assay was based on the capacity of the extract to inhibit the photochemical reduction of Nitroblue tetrazolium (NBT)[24] in the presence of the riboflavin-light-NBT system. In this reaction each 3 ml reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 2 µM riboflavin, 100 µM Ethylene diamine tetra acetic acid (EDTA), NBT (75 µM) and different concentration of test sample solution. It was kept in front of fluorescent light and absorbance was taken after 6 min at 560 nm by using a Deep Vision (1371) UV–Vis Spectrophotometer. Identical tubes with reaction

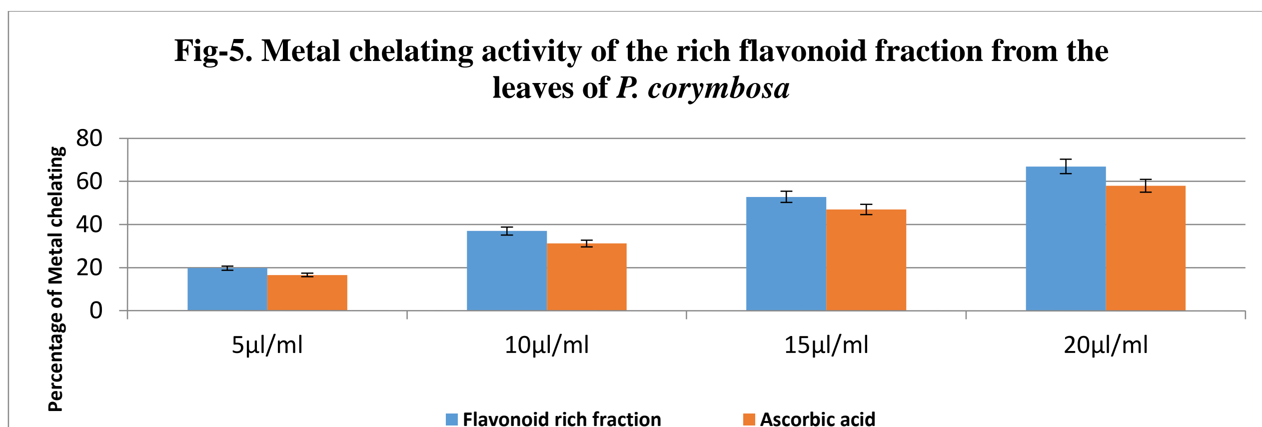








mixture were kept in the dark and served as blanks. The capability of scavenging superoxide radical was calculated



using the following equation

% Super oxide radical scavenging capacity= $[(A_0- A_1)/A_0] \times 100$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of flavonoid rich fraction or standard Ascorbic acid

Nitric oxide radical scavenging activity

The ability of flavonoid rich fraction to scavenge nitric oxide radical was determined by the method described by Olabinri et al.[25] 0.1ml of sodium nitroprusside (10mM) in phosphate buffer (0.2 M, pH 7.8) was mixed with different concentration of flavonoid rich fraction and incubated at room temperature for 150 min. After incubation period, 0.2 ml of Griess reagent (1% Sulfanilamide, 2% Phosphoric acid and 0.1% N- (1-Naphthyl) ethylene diamine dihydrochloride) was added. The absorbance of the reaction mixture was read at 546 nm against blank. All readings were taken in triplicate and Ascorbic acid was used as the standard. The % inhibition was calculated by following equation. The EC_{50} values and percent inhibition by various concentration of flavonoid rich fraction were calculated by comparing the absorbance values of the control and test compounds.

% Nitric oxide radical scavenging capacity= $[(A_0- A_1)/A_0] \times 100$

Where A_0 is the absorbance of control and A_1 is the absorbance of flavonoid rich fraction or standard Ascorbic acid.

Metal chelating activity

The chelating of ferrous ions by flavonoid rich fraction and standards were estimated by the method of by Iihami et al.[26] Briefly 1ml of different concentrations of methanol extract was added to 0.05ml of 2 mM ferric chloride solution. The reaction was initiated by the addition of 0.2 ml of 5 mM Ferrozine and the mixture was shaken vigorously. After 10 min, the absorbance of the solution was measured at 562 nm against blank. All readings were taken in triplicate and Ascorbic acid was used as the standard. The percentage of inhibition of ferrozoine- Fe^{2+} complex was calculated by following equation. The EC_{50} values and percent inhibition by various concentration of flavonoid rich fraction were calculated by comparing the absorbance values of the control and test compounds.

% Inhibition of ferrozoine- Fe^{2+} complex = $[(A_0- A_1)/A_0] \times 100$

Where A_0 is the absorbance of control and A_1 is the absorbance of flavonoid rich fraction or standard Ascorbic acid. The control does not contain $FeCl_2$ and ferrozine, complex formation molecules.

ABTS assay on TLC

This procedure was applied for flavonoid rich fraction of *P. corymbosa*. Five microlitre of 1:10 dilution of the flavonoid rich fraction was applied to the TLC plate and Toluene: Tetrahydrofuron: Acetic acid (9.5:2.5:0.4) mixture was used as developer. The plate was sprayed with 0.2% ABTS reagent in methanol and left at room temperature for 30 min. White spots were formed from bleaching of the green colour of ABTS reagent, and was evaluated as positive antioxidant activity.

GC–MS analysis

The rich flavonoid fraction was analyzed by GC–MS (QP-2010, Shimadzu Co., Kyoto, Japan) equipped with 30-m×0.25 mm DB-5MS column (Agilent Technologies, J & W Scientific Products, Folsom, CA). The carrier gas was helium. The temperature program was set as follows: 100 ^{0}C hold for 5 min, raised at 4 ^{0}C/min to 280 ^{0}C, and hold for 5 min. The injector and detector temperatures were set at 250 and 280 ^{0}C, respectively. The ion source and interface temperatures were set at 200 and 250 ^{0}C, respectively. The mass range was scanned from 50 to 900 amu. The control of the GC–MS system and the data peak processing were controlled by means of Shimadzu's GC–MS solution software, version 2.4. Compound identification was verified based on the relative retention time and mass fragmentation pattern spectra with those of standards and the NIST147. LIB. database of the GC–MS system (Shimadzu).

Statistical analysis

The antioxidant assays were ascertained using one way analysis of variance (ANOVA). Furthermore, Duncan's post hoc test was applied, so as to determine the statistically significant different values. All statistical handling was performed using SPSS software, v. 14.0 (SPSS, Chicago, Ill., U.S.A.

RESULTS AND DISCUSSION

Total phenolic and total flavonoid content

The content of phenolic compounds in *P. corymbosa* flavonoid rich fraction was determined through a linear gallic acid standard curve (y = 8.2313x + 0.078; r2 =

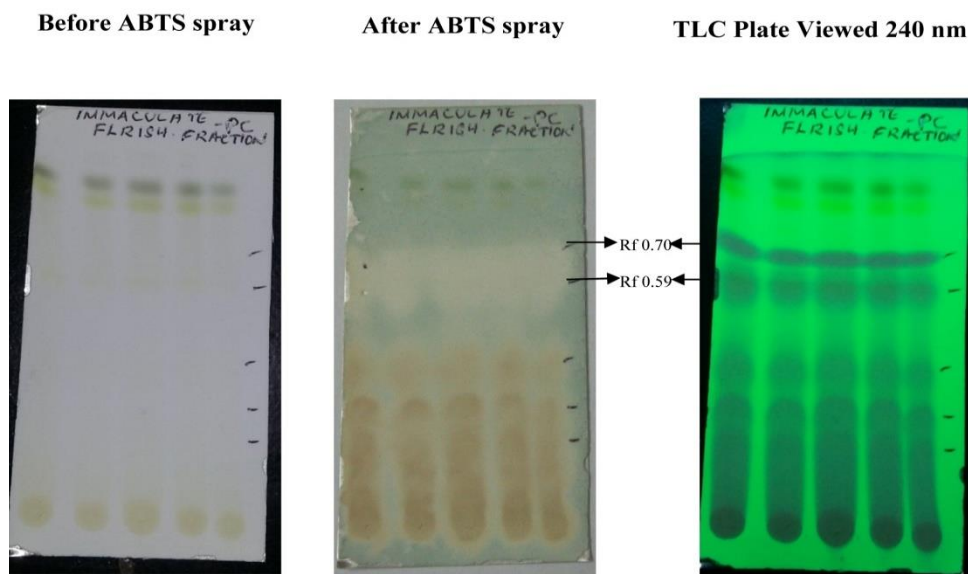


Figure 6: ABTS assay on TLC

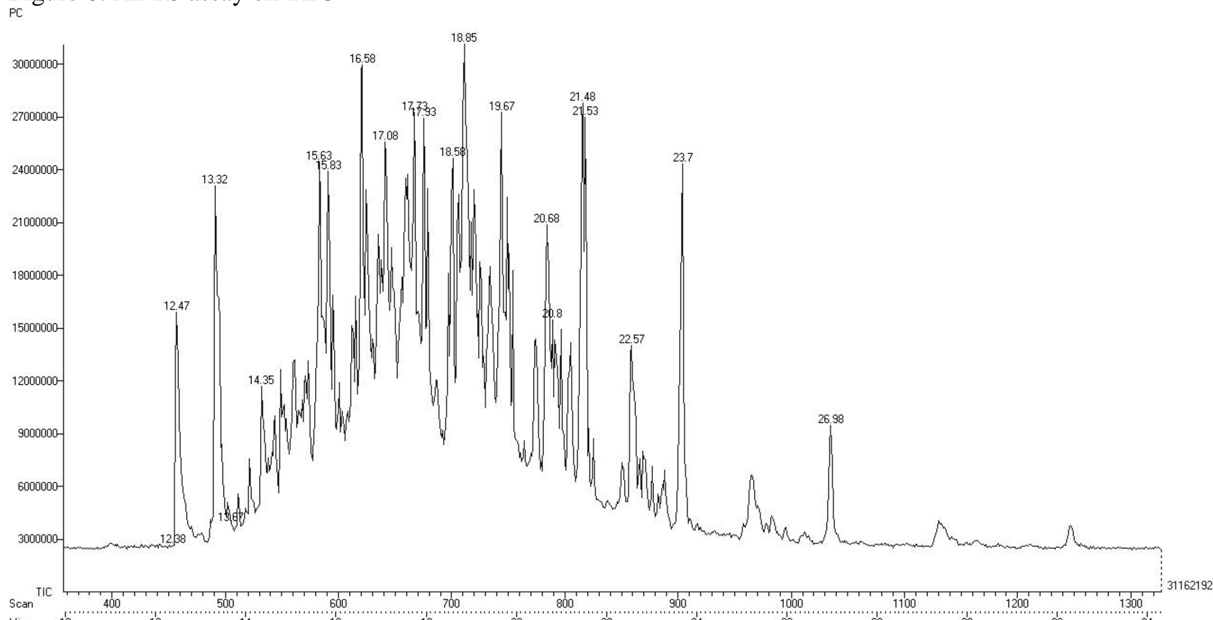


Figure 7: GC–MS chromatogram of Flavonoid rich fraction from the leaves of *P. corymbosa*. 1, Catechol; 2, 1-Hexadecanol;3, Phenol,2-6 dimethoxy; 4, 1-Decanol,2-hexyl; 5,Benzaldehyde, 2 hydroxy-3-methoxyl; 6, Oxirane; 7, Isovanillic acid; 8, 10-Nonadecanone; 9, 13,14-Epoxy,tetradec-11-en-1-ol acetate; 10, Cis-9-Tricosene.

0.9971). The total phenolic content of flavonoid rich fraction was 36.8±13.00 mg GAE/g extract and was statistically significant ($p < 0.05$). This finding is in agreement with some previous studies that reported the total phenolic content of leaf extract was higher than other parts of the plant for *Cucumis melo* and *Coriandrum sativum* [27, 28]. This suggests that leaf might be the part that is rich in phenolic compounds in many plants. Several studies have revealed that the phenolic content in the plants are associated with their antioxidant activities, probably due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers[29]. The total flavonoid content of flavonoid rich fraction from *P. corymbosa* was evaluated by aluminium colorimetric assay. Rutin was used as a standard ($y = 0.0097x + 0.0127$, r2 = 0.9995) and the total flavonoid content of *P. corymbosa* flavonoid rich fraction were expressed in microgram of rutin equivalents per gram of extract (µg RE/g extract). The total flavonoid content of flavonoid rich fraction of *P. corymbosa* was 78.2±1.23 µg RE/g extract ($p < 0.05$). This indicates that the flavonoids are the major phenolic compounds present in *P. corymbosa* plant. These data were in accordance with previous research[30], showed that the high total flavonoid content increases antioxidant activity and there was a linear correlation between phenolic content and antioxidant activity.

Table 1: Analysis of flavonoid rich fractions by GC–MS

S.No	Compound	Retention Time(min)	Molecular weight	Major peaks
1.	Catechol	12.47	110	107,91,74,57
2.	1-Hexadecanol	13.32	242	111,97,84,70,55
3.	Phenol,2-6 dimethoxy	15.63	154.16	139,111,93,65
4.	1-Decanol,2-hexyl	15.83	242.44	154,125,111,97,83
5.	Benzaldehyde,2hydroxy-3-methoxyl	16.58	197	152,122,106,81
6.	Oxirane	17.08	149	139,125,83,71
7.	Isovanillic acid	17.82	168.15	153,125,97,79
8.	10-Nonadecanone	18.58	197	117,155,125,85
9.	13,14-Epoxy,tetradec-11-en-1-ol acetate	18.85	268.3	180,149,111,97
10.	Cis-9-Tricosene	19.67	322	196,167,139,111
11.	1-Tetracosanol	23.7	354.6	308,252,209,153
12.	2-methyl-E-7-hexadecene	22.57	238.4	238,223,210,168
13.	1-Eicosanol	21.53	298.5	308,280,252,224,196
14.	Cyclodocosane,ethyl	20.68	196.3	307,181,167,83

Reducing power assay

The reducing power capacity of the flavonoid rich fraction of the *P. corymbosa* was measured by their capability to transform $Fe^{3+}$to Fe^{2+}at various concentrations (5, 10, 15 and 20 µg/mL). The results revealed that the reducing activity significantly increased as the concentration of the flavonoid rich fraction increased (Fig-1). The reducing power activity of the flavonoid rich fraction of the *P. corymbosa* data are presented as the means ± SEM of triplicates $p < 0.001$ from the control. The reductive capacity of a compound depends on the presence of reductones, which exhibit antioxidative potential by breaking the free radical chain and donating a hydrogen atom. Therefore, reducing activity leads to the termination of the radical chain reactions that may otherwise be very damaging[31]. The presence of antioxidant reluctant in the flavonoid rich fraction of *P. corymbosa* causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form, indicating that the polyphenolic extract of *T. chebula* has significant reducing power.

ABTS radical activity

ABTS radical cation decolourisation assay, showed quite similar results compared to Ascorbic acid. The flavonoid rich fraction of the *P. corymbosa* fully scavenged ABTS (the absorption after 6 min was close to 0). In ABTS assays, the EC_{50} values in flavonoid rich fraction of *P. corymbosa* was lower than pure ascorbic acid being 13.78 and 58.28 µg/ml, respectively (Fig-2). However, the agreement between this assays, in our study, probably indicated that the activity was mainly due to phenolics and flavonoids. It also should be noted that the reaction with ABTS was quite fast and almost in all cases was completed in 0.25–0.5 min. During the remainder of the reaction time the changes in absorption were negligible. The results provided in Fig-2 demonstrated that the most active radical scavengers were the flavonoid rich fraction and was statistically significant ($p < 0.05$). It is known that only flavonoids of a certain structure and particularly hydroxyl position in the molecule determine antioxidant properties; in general these properties depend on the ability to donate hydrogen or electron to a free radical[30].

Superoxide anion scavenging activity

The flavonoid rich fraction of the *P. corymbosa* exhibited potent scavenging activity for superoxide radicals in a concentration dependent manner. The decrease of absorbance at 560 nm with antioxidants indicated the consumption of superoxide anion in the reaction mixture[32]. Fig. 3 shows the percentage inhibition of superoxide radical generation by 5 to 20 µl/ml of flavonoid rich fraction and it was compared with Ascorbic acid. The flavonoid rich fraction exhibited higher superoxide radical scavenging activity (EC_{50} 19.39) than Ascorbic acid (EC_{50} 20.50) ($P < 0.01$). The percentage inhibition of superoxide generation by 20 µl/ml amount of flavonoid rich fraction was found as 69% and greater than that of some doses of Ascorbic acid (63%). Plant possess polyphenols that bring forth protective effects by scavenging superoxide, which is implicated in tissue damage and accelerated inactivation of vasorelaxing nitric oxide. Preventing the interaction of superoxide with tissue biomolecules depends not only on the extent of superoxide scavenging but also on scavenging velocity[33].

Nitric oxide radical scavenging assay

In the present study, the nitric oxide radical quenching activity of the flavonoid rich fraction from the leaves of *P. corymbosa* was detected and compared with the standard ascorbic acid. The flavonoid rich fraction exhibited the maximum inhibition of 68% at a concentration of 20 µg/mL, with an EC_{50} value of 12.55 µg/mL, in a concentration-dependent manner when compared to ascorbic acid was 15.67 µg/mL (Fig-4). The scavenging activity of the extract against nitric oxide was detected by its ability to inhibit the formation of nitrite through direct competition with oxygen and oxides nitrogen in the reaction mixture[31]. The decrease in the concentration of the nitric oxide radical activity was significant in *P.*

corymbosa than standard. This may be due to the presence of antioxidant flavonoid molecules.

Metal chelating activity

As shown in Fig-5, the formation of the Fe^{2+} ferrozine complex was not completed in the presence of flavonoid rich fraction of the *P. corymbosa*, indicating that extract chelates the iron. The absorbance of Fe^{2+} ferrozine complex was linearly decreased dose-dependently (from 5 to 20 µl/ml). The difference between flavonoid rich fraction of the *P. corymbosa* and the control was statistically significant ($P < 0.01$). The metal chelating capacity in flavonoid rich fraction of the *P. corymbosa* and ascorbic acid were found` as EC_{50} 14.44 and 41 µl/ml, respectively. Metal chelating capacity is important as it reduced the concentration of the catalyzing transition metal in lipid peroxidation[34]. It was reported that chelating agents, form bonds with a metal, are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion[35].

ABTS assay on TLC

The flavonoid rich fraction was applied on silica gel TLC plates and two main zones (Rf Value 0.70 and 0.59) appeared after spraying of ABTS for the identification of antioxidant molecules (Fig-6). Although isolation and the structural determination have not yet been completed, preliminary results showed that these components are flavonoids.

Analysis of flavonoid rich fractions by GC–MS

The flavonoid rich fraction of the *P. corymbosa* contained the highest flavonoid content and showed the strongest antioxidant activity. It was therefore analyzed by GC–MS to determine its chemical composition that may contribute to this activity. The GC–MS analysis showed a variety of phenolic compounds (Fig-7). By comparing the MS spectral data with those of standards and MS library, 14 flavonoid compounds were identified (Table-1). Of which, the major compounds are Catechol; 1-Hexadecanol; Phenol,2-6 dimethoxy; 1-Decanol,2-hexyl; Benzaldehyde, 2 hydroxy-3-methoxyl; Oxirane; Isovanillic acid; 10-Nonadecanone; 13,14-Epoxy,tetradec-11-en-1-ol acetate; Cis-9-Tricosene.

CONCLUSION

The flavonoid rich fraction of *P. corymbosa* can protect the body from oxidative stress of ROS, which may be due to the presence of phytochemicals in the form of flavonoids compounds present in this plant and it may contribute to their medicinal properties and may be used in nutraceuticals and in food industry. However, additional studies are necessary to develop a method for the fractionation and identification of most active antioxidant molecules and thereby can be used in the prevention and treatment of ageing related diseases and can be considered as good source for drug discovery.

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REFERENCES

1. Van Wyk BE, Wink M. Medicinal Plants of the World. Timber Press. Portland, OR, Cambridge, 2004.
2. Addae-Mensah I. Plant biodiversity, herbal medicine, intellectual property rights and industrially developing countries: socio-economic, ethical and legal implications. In: Lauer H, editor. Ghana: changing values/changing technologies: Ghanaian philosophical studies, II. Washington DC: The Council for Research in Values and Philosophy, 2000.
3. Das AK, Dutta BK, Sharma GD. Medicinal plants used by different tribes of Cachar district, Assam. Indian J Tradit Knowl 2008; 7: 446-454.
4. Muthu C, Ayyanar M, Raja N, Ignacimuthu S. Medicinal plants used by traditional healers in Kancheepuram District of Tamil Nadu, India. J Ethnobiol Ethnomed 2006; 2: 43.
5. Gacche RN, Kabaliye VN, Dhole NA, Jadhav AD. Antioxidant potential of selected vegetables commonly used in diet in Asian continent. Indian J Nat Prod Resour 2010; 1: 306-313.
6. Shanmugaraj BM, Ramamoorthy D, Aziz R, Ramalingam S. Antioxidant Perspective of selected micinal plants in India: A probable source of natural antioxidants. J Pharmacy Res 2013; 7: 271-274.
7. Lin Y, Fiskum G, Schubert D. Generation of reactive oxygen species by mitochondrial electron transport chain. J Neurochem 2002; 80: 780-787.
8. Avonti BT, Nazm S, Cadi PB, Mohiduzzaman M. Antioxidant capacity and total phenolic contents in hydrophilic extracts of selected Bangladeshi medicinal plants. Asian Pac J Trop Med 2014; 7(1): S568-S573.
9. Yoo MK, Lee HC, Lee H, Moon B and Lee YC. Reactive antioxidant and cytoprotective activities of common herbs. Food Chem 2008; 106: 929-936.
10. Reuter S, Gupta SC, Chaturved MM, Aggarwa BB. Oxidative stress, inflammation, and cancer: how are they linked? Free Radic Biol Med 2010; 49(11): 1603-1616.
11. Li H, Horke S, Forstermann U. Oxidative stress in vascular disease and its pharmacological prevention. Trends Pharmacol Sci 2013; 34(6): 313-9.
12. Cencioni C, Spallotta F, Martelli F, Valente S, Mai A, Zeiher AM. Oxidative stress and epigenetic regulation in ageing and age-related diseases. Int J Mol Sci 2013; 14(9): 17643-17663.
13. Abu AR,Zuraini Z, Lacimanan YL, Sreenivasan S. Antioxidant activity and phytochemical screening of methanol extracts of *Euphorbia hirta* L. Asian Pac J Trop Med 2011; 386 -390
14. Molyneux P. The use of stable free radical diphenylpicrylhydrazyl(DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol 2004; 26(2): 211-219.

15. Ramamoorthy PK, Bono A. Antioxidant activity, total phenolic and flavonoid content of *Morinda citrifolia* fruit extracts from various extraction processess. J Eng Sci Technol 2007; 2: 70-80.
16. Jiangning G, Xinchu W, Hou W, inghua L, Kaishnu B. Antioxidants from Chinese medicinal herbs. Food Chem 2004; 88: 347-350.
17. Yazdanparast R, Bahramikias S, Ardestani A. *Nasturtium officinale* reduces oxidative stress and enhances antioxidant capacityin hypercholesterolaemic rats, Chem Biol Interact 2008; 172: 176–184.
18. Djeridane A, Yousfi M, Boutassouna D, Stocher P, Vidal N. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem 2006; 97: 654-660.
19. Quettier-Deleu C, Gressier B, Vasseur J, Dine T, Brunet C, Luyckx M. Phenolic compounds and antioxidant activities of buckwheat (Fagopyrum sculentum Moench) hulls and flour. Journal of Ethnopharmacology 2000; 72: 35–42.
20. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorizing assay. Free Radicals in Biology and Medicine 1999; 26: 1231-7.
21. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Japanese Journal of Nutrition 1986; 44: 307–315.
22. Tripathi YB, Pandey Ekta. Role of Alcoholic extract of shoot of *H. perforatum* (Lim) on LPO and various species of free radicals in Rats. Indian Journal of Experimental Biology 1999; 37: 567-571.
23. Tripathi YB, Sharma M. The Interaction of *R. cordifolia* with iron redox status: mechanistic aspects in FR reactions. Phytomedicine 1999; 6 (1): 51-7.
24. Beauchamp C, Fridovich I. Superoxide dismutase: improved assay and an assay applicable to acrylamide gels. Analytical Biochemistry1971; 44: 276-87.
25. Olabinri BM, Odedire OO, Olaleye MT, Adekunle AS, Ehigie LO, Olabinri PF. In vitro evaluation of hydroxyl and nitric oxide radical scavenging activities of artemether. Research Journal of Biological Science. 2010; 5(1): 102-5.
26. Iihami G, Emin BM, Munir O, Irfan KO. Antioxidant and analgesic activities of turpentine of *Pinus nigra Arn subsp pallsian A* (Lamb) Holmboe. Journal of Ethnopharmacology 2003; 86: 51-58.
27. Hajar II, Kim WC, Abdalbasit AM, Maznah I. Phenolic content and antioxidant activity of cantaloupe (*Cucumis melo*) methanolic extracts. Food Chemistry 2010; 119: 643–647.
28. Peter YYW, David DK. Studies on the dual antioxidant and antibacterial properties of parsley (*Petroselinum crispum*) and cilantro (*Coriandrum sativum*) extracts. Food Chemistry 2006; 97: 505-515.
29. Chang ST, Wu JH, Wang SY, Kang PL, Yang NS, Shyur LF. Antioxidant activity of extracts from *Acacia confusa* bark and heartwood. Journal of Agricultural & Food Chemistry 2001; 49(7): 3420–3424.
30. Miliauskas G, Venskutonis PR, van Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chemistry 2004; 85: 231–237.
31. Alasalvar C, Karamac M, Amarowicz R, Shahidi F. Antioxidantand antiradical activities in extracts of hazelnut kernel (*Corylusa vellana* L.) and hazelnut green leafy cover. J Agric Food Chem 2006; 54: 4826-4832.
32. Oktay M, Gulcin I, Kufrevioglu OI. Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. Lebensmittel-Wissenchaft und Technologie 2003; 36: 263-271.
33. Dirk T, Thomas B, Andreas L, Petra C, Steffi H, Reinhard B, Wolfgang K, Renate R. Reaction rate constants of superoxide scavenging by plant antioxidants. Free Radical Biology and Medicine 2003; 35 (12): 1599-1607.
34. Duh PD, Tu YY, Yen GC. Antioxidant activity of water extract of Harug Jyur (*Chrysanthemum morifolium* Ramat). Lebensmittel-Wissenchaft und Technologie 1999; 32: 269–277.
35. Gordon MH. The mechanism of the antioxidant action in vitro. In: Hudson, B.J.F. (Ed.), Food Antioxidants 1990; Elsevier, London, 1–18.