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QUANTIFICATION OF PHENOLIC COMPOUNDS, *IN VITRO* ANTIOXIDANT ANALYSIS AND SCREENING OF CHEMICAL COMPOUNDS USING GC-MS IN *ACALYPHA ALNIFOLIA* KLEIN EX WILLD. - A LEAFY VEGETABLE

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ABSTRACT

Revealing antioxidant potential of *Acalypha alnifolia* leaf extracts and quantification of its phenolic compounds are the main objectives. Preliminary phytochemical analysis has been carried out by using standard methods. Exemplary reducing and radical scavenging antioxidant activity have drawn closer and taken notice by *in vitro* assays such as FRAP, Phosphomolybdenum reducing power assays, DPPH, Superoxide and ABTS radical scavenging assays. GCMS have been undertaken for screening compounds. Quantification of phenolic compounds shows acetone leaf extract having much more than other solvent extracts. The results show acetone and methanol leaf extracts having commendable antioxidant activity than other extracts. Nine different compounds were identified from the acetone extracts when taken for GCMS. As per the previous reports, few of them possessing pharmacological importance are tabulated. This study winds up the antioxidant potency of this leafy vegetable revealing the presence of pharmacologically important compounds make them considerable for health supplements.

KEYWORDS: ***Acalypha alnifolia,*** **Leafy vegetable, Phenolic compounds, Antioxidant, GCMS**

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INTRODUCTION

Reactive oxygen species (ROS) are continuously produced during cell metabolism and under normal conditions; they are scavenged and converted to nonreactive species by different intracellular enzymatic and non-enzymatic antioxidant system [1]. It is possible to reduce the risks of chronic diseases and prevent disease progression by either enhancing the body's natural antioxidant defenses or by supplementing with proven dietary antioxidants [2]. Thus, a practical way to control these diseases is to increase the dietary intake of fruits and vegetables, which are rich sources of antioxidants [3,4,5]. Since, the plant kingdom offers a wide range of natural antioxidants; recently there has been considerable interest in finding natural antioxidants from plant materials to replace synthetic ones. Natural antioxidants occur in all higher plants, and in all parts of the plants[6]. Antioxidant compounds in food play an important role as a health-protecting factor. Natural antioxidant substances are accepted to be secure and sound from the time when they occur in plant foods, and more enviable than synthetic one.

There is growing awareness in correlating the phytochemical constituent of medicinal plants with its pharmacological activity[7-13]. The Plant *Acalypha alnifolia* belongs to the family Euphorbiaceae containing 60 genera and the Genus Acalypha having 23 species in it. As *A. alnifolia* is one of the endemic plants of Nilgiris, its leaf is commonly used as leafy vegetable by the local people [14]. The Irula tribes of Marudhamalai hills have been used this plant to treat dysentery [15]. Villagers of Dharapuram taluk use the *A. alnifolia* leaf juice mixed with 150 ml boiled cow milk twice a day for five months against diabetes [16]. Generally, any part of the plant can be used for antioxidant studies but most commonly used part is leaf followed by fruit [17]. In addition to that, the leaf is being used as a leafy vegetable. Hence, the leaf is taken for this study. As far as known, very few pharmacological studies have been conducted in *A. alnifolia* except larvicidal activity. The aim of our present study is to evaluate the antioxidant properties and the quantification of phenolic compounds by GCMS was undertaken. Though searching of new or alternatives for prevailing health problems is an ultimate aim of scientists and also a step to make a focal point of this ethnobotanically important unexplored plant.

MATERIALS AND METHODS

Plant collection

The plant *A. alnifolia* leaves were collected from Bharathiar University campus and was authenticated by Botanical Survey of India, Southern circle, Coimbatore. The voucher specimen was deposited in Department of Botany, Bharathiar University and BSI Coimbatore. The collected leaves were washed in running tap water and shade dried. The dried leaves are powdered and stored in an air tight container for further use.

Qualitative Phytochemical Screening

The leaf of the plant extracts were analyzed for the presence of major phytochemicals such as Carbohydrates, Proteins, Amino acids, Alkaloids, Saponins, Phenolic compounds, Tannins, Flavonoids, Glycosides, Flavonol glycosides, Cardiac glycosides, Phytosterols, Fixed oils & fats, and Gums & mucilages according to standard methods [18].

Quantification of total Phenolics

Total phenolic compound contents were determined by the Folin-Ciocalteau method[19]. The sample extracts (50 µl of different solvent extracts) were mixed with Folin Ciocalteu reagent (0.5 ml, 1:1 diluted with distilled water) for 5 min and aqueous Na_2CO_3 (2.5 ml, 20%) was added. The mixture was vortexed, allowed to stand for 40 min at room temperature in dark and the phenols were determined by colorimetric method at 725 nm. The standard curve was prepared by 0, 5, 10, 15, 20 and 25 mg/ ml solutions of Gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of Gallic acid equivalent.

Quantification of Free Phenolics

Free Phenolics were estimated as per Siddhuraju and Manian[20] method with some modification; 100 mg of PVPP was weighed, and 500 µl each of distilled water and sample extracts was added. The content was vortexed and kept in the test tube at 4ºC for 4 hrs. Then the sample was centrifuged at 4,000 rpm for 10 min at room temperature, and the supernatant was collected. The phenolic content of the supernatant was measured and expressed as the content of non-tannin phenolics on dry matter basis. From the above results, the tannin content of the sample was calculated.

Quantification of Flavonoids

The flavonoid contents estimated as per Zhishen *et al*. [21]. Initially, 500 µl of all the plant extracts were taken in different test tubes. To each extract, 2 mL of distilled water was added. Then 150 mL of 5% $NaNO_2$ was added to all the test tubes followed by incubation at room temperature for 6 min. After incubation, 150 mL of $AlCl_3$ (10%) was added to all the test tubes including the blank. All the test tubes were incubated for 6 min at room temperature. Then 2 mL of 4% NaOH was added, which was made up to 5 mL using distilled water. The contents in all the test tubes were vortexed well, and were allowed to stand for 15 min at room temperature. The pink color developed because of the presence of flavonoids was read spectrophotometrically at 510 nm. Rutin was used for the calibration curve.

Extraction of Plant material

The powdered plant leaf was packed in small thimbles separately and extracted successively with different solvents such as petroleum ether, chloroform, acetone and methanol in the increasing order of polarity using soxhlet apparatus. The extract obtained was used for the assessment of various antioxidant assays and for further analysis.

Antioxidant assays:DPPH radical scavenging assay

The antioxidant activity of the extract was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH, according to the method of Blios[22]. Sample extracts at various concentrations were taken and the volume was adjusted to 100 µL with methanol. About 3 mL of 0.1 mM methanolic solution of DPPH was added to the aliquots of samples and shaken vigorously. The tubes were allowed to stand for 20 minutes at 27°C. The absorbance of the sample was measured at 517 nm against the blank. Radical scavenging activity of the samples was expressed as IC_{50} which is the concentration of the sample required to inhibit 50% of $DPPH^{\cdot}$ concentration.

Phosphomolybdenum

The phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, α-tocopherol, and carotenoids. The antioxidant activity of samples was evaluated by the phosphomolybdenum method[23]. An aliquot of 100µl of sample solution (1 mM in dimethyl sulfoxide) was combined in a 4-mL vial with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials were capped and incubated in a water bath at 95ºC for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The results reported are mean values expressed as grams of ascorbic acid equivalents per gram sample.

Ferric Reducing Antioxidant Power

The antioxidant capacities of phenolic extracts of samples were estimated according to the procedure described by Pulido *et al*., [24]. The ferric-reducing antioxidant power (FRAP) reagent contained 2.5 mL of 20 mmol/L TPTZ (2,4,6- tripyridyl-*s*-triazine) solution in 40 mmol/L HCl and2.5 mL of 20 mmol/L $FeCl_3.6H_2O$ and 25 mL of 0.3 mol/L acetate buffer (pH 3.6) described by Siddhuraju and Becker (22). FRAP reagent (900 mL), prepared freshly and incubated at 37ºC, was mixed with 90 mL of distilled water and 30 mL of test sample or methanol (for the reagent blank). The test samples and reagent blank were incubated at 37ºC for 30 min in a water bath. At the end of incubation, the

absorbance readings were taken immediately at 593 nm. The FRAP value is expressed as mmol Fe (II) equivalent/mg extract.

ABTS (2, 2 – Azinobis (3- ethyl-benzothiazoline-6- sulphonic acid) radical scavenging assay

The total antioxidant activity was measured by ABTS radical cation decolouration assay according to the method of Re *et al*., [25]. ABTS was produced by reacting 7mM ABTS aqueous solution with 2.4 mM potassium persulfate in the dark for 12-16 h at room temperature. Prior to assay, this solution was diluted in ethanol (about 1:89v/v) and equilibrated at 30º C to give an absorbance of 0.70±0.02 at 734 nm. After the addition of 1ml of diluted ABTS solution to the sample or Trolox incubated at 30º C for 30 minutes. The percentage inhibition was calculated against the blank and absorbance at 734 nm. The unit of total antioxidant activity is defined as the concentration of Trolox having equivalent antioxidant activity expresses as µM/g sample extracts.

Superoxide radical scavenging activity

The assay was based on the capacity of the methanol extract to inhibit formazan formation by scavenging superoxide radicals generated in riboflavin–light–NBT system [26]. Each 3mL reaction mixture contained 50 mM sodium phosphate buffer (pH 6), 20 µg riboflavin and 12 mM EDTA, and 0.1 mg NBT. Reaction was started by illuminating the reaction mixture with different concentrations of sample extracts for 90 s. Immediately after illumination, the absorbance was measured at 590 nm. The percentage inhibition of superoxide anion generation was calculated.

% inhibition = [(Control OD- Sample OD)/ Control OD] x 100

GC-MS analysis

The GC – MS analysis was carried out using a Clarus 500 Perkin – elmer (Auto system XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perkin Elmer Turbomass 5.1 spectrometer with an Elite – 1 (100% Dimethyl poly siloxane), 30m x 0.25 mm ID x 1µm of capillary column. The instrument was set to an initial temperature of 110ºC, and maintained at this temperature for 2 min. At the end of this period the oven temperature was rose up to 280ºC, at the rate of an increase of 5ºC/min, and maintained for 9 min. Injection port temperature was ensured as 250ºC and Helium flow rate as one ml/min. The ionization voltage was 70eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45-450 (m/z). Spectrum matches with the stored database of NIST (National Institute of Standard and Technology) library.

Statistics

Results were statistically analyzed and values expressed as mean± standard deviations.

RESULTS

Preliminary phytochemical analysis (qualitatively)

Standardized methods are followed to find the compounds for the presence or absence qualitatively. Phytochemical screening of *A. alnifolia* leaf was undertaken; Shade dried and powdered plant leaf was macerate with water; extract was taken for analysis. Table 1 shown the presence of carbohydrate, cardiac glycosides, phenolic compounds found ideal and proteins, aminoacids, phytosterols was found moderately.

Table 1
Qualitative phytochemical screening of A. alnifolia leaf

Compounds	Presence or Absence
Carbohydrate	+++
Proteins	++
Amino acids	++
Alkaloids	--
Saponins	--
Phenolics	+++
Tannins	++
Flavonoids	+++
Glycosides	--
Phytosterols	++
Flavanol glycosides	--
Cardiac glycosides	+++
Fixed oils and fat	--
Gums and mucilages	--

+ present; ++ moderately present; +++ luxuriantly present ; -- Absent.

Yield Percentage

The successive solvent extract yield percentage was shown in the table 2. The non polar to polar solvents were yield according to the compound present in the sample dissolves in it. The solvents with different polar nature of following five were used for successive solvent extraction such as petroleum ether, chloroform, acetone, methanol and Hot water. The yield percentage was measured by the weight obtained at each solvent extraction. Methanol (16%) was yielded higher than acetone (9.66%). Petroleum ether and chloroform yield was very least where both were low polar solvents.

Table. 2
Different solvents extract yield in percentage.

Solvents	Yield %
Petroleum ether	3.33
Chloroform	2
Acetone	9.66
Methanol	16
Hot water	7.5

Quantification

The quantification of total phenols, flavonoids and tannins are in table 3. The result shows as Gallic acid equivalent per gram. Acetone and Methanol extracts of *A. alnifolia* leaf having significant phenol content especially, acetone extract shows higher (35.97) than other extracts. Flavonoids are concordantly the same in all the extracts except acetone (18.2) which was very good. It was noted that the acetone is best suitable solvent for flavonoid extraction. Likewise the free phenolics are estimated to measure tannin content in the sample. Tannins were recorded very less in petroleum ether and chloroform extracts. Acetone and methanol extracts outlined the tannin content in *A. alnifolia* leaf as 28.62 and 18.39 mg GAE/g respectively.

Table 3
Phenols, Flavonoids and tannin content Of Acalypha alnifolia leaf extracts.

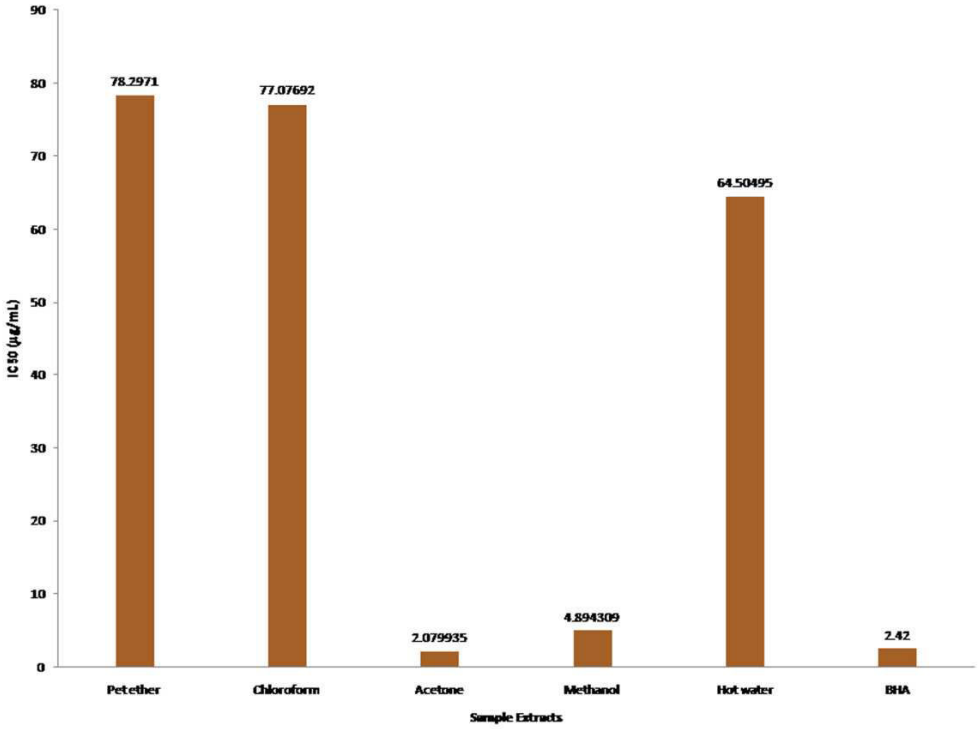
Extracts	Total Phenolics (mg GAE/g)	Flavonoids (mg RE/g)	Tannin (mg GAE/g)
Petroleum ether	4.67 ± 0.111	8.3±0.62	0.448148±0.05
Chloroform	4.57 ± 0.084	8.1±0.7	0.118519±0.13
Acetone	35.97 ± 0.589	18.2±0.7	28.62805±0.70
Methanol	24.33 ± 0.549	8.67±0.15	18.39957±1.09

GAE- Gallic acid Equivalence; ±- standard Deviation

DPPH radical scavenging activity

DPPH is a radical which was committed with our extracts separately and in different concentration. The hydrogen donated by the antioxidants in the sample and decreased the colour of DPPH. The IC_{50} was estimated where how much least concentration of sample was enough to reduce the radicals concentration as half. BHA was taken as control and the results are shown in figure 1. It shows 2.07 mg/ml of acetone extract is having enough potency to inhibit the fifty percent free radicals which was twice better than methanol leaf extract. The other solvent extracts were not much good like these two.

Figure 1
DPPH radical scavenging activity of A. alnifolia leaf extract



Phosphomolybdenum Reducing Power Assay

The different solvent extracts implemented to this assay and the result was shown in table 4. The antioxidant present in the sample which was reduced the Mo VI and forms green colour. The results in the table were mg ascorbic acid equivalent per gram. Out of 5 different extracts with their polarity, the methanol extract was better than other solvents. Acetone was good but next to the methanol. Chloroform and hot water extracts were concurrently similar with meager difference.

Ferric Reducing Antioxidant Power Assay (FRAP)

Ferric reducing antioxidant power of the sample extracts was confirmed by the formation of blue colour due to the reducing capacity and its colour density increased which depends on the concentration of the sample. The reducing capacity was estimated by the absorbance and results were shown in mmol equivalence of Fe (II) per mg. The acetone extract was considering higher (324.10 mmol/mg) which was meager difference with methanol extract (323.43 mmol/mg). Hot water extract was better than Chloroform and petroleum ether extracts.

ABTS radical scavenging activity

The ABTS radicals were scavenged by the antioxidants present in the extract and cause the decoloration and which was concentration dependent. This activity of different extracts is given in the table 4. Even the phenolic compounds quantity were considerably more in acetone extract, the ABTS radical scavenging activity is higher in Methanol extract. The petroleum ether leaf extracts having very low scavenging activity when compare to other solvent extracts. The results have presented as µg Equivalence of Trolox per gram. The results of phosphomolybdenum, FRAP activity and ABTS scavenging ability were shown in table 4.

Table 4
Results of antioxidant assays namely phosphomolybdenum, FRAP activity and ABTS scavenging ability

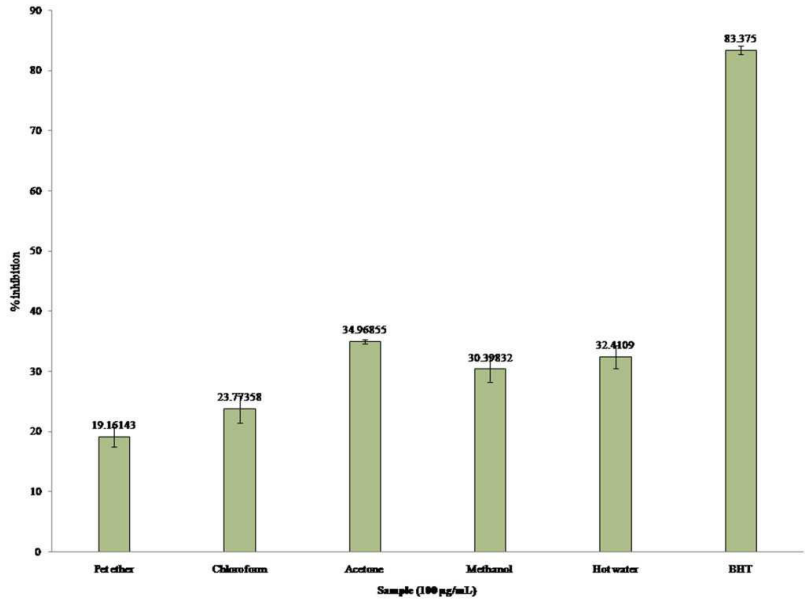
Antioxidant assays / Extracts	Phosphomolybdenum mg Ascorbic acid Equivalence/g	FRAP Mmol Equivalence of Fe (II) /mg	ABTS µg Equivalence of Trolox/g
Petroleum ether	38.69732±2.163	31.98068±0.22	973.5686
Chloroform	83.98467±4.265	51.5942±0.80	3906.703
Acetone	104.8659±4.087	324.1063±0.16	39854.17
Methanol	139.6935±2.816	323.43±0.72	45902.73
Hot water	82.87356±6.386	146.9082±0.76	8078.548

±- standard Deviation

Superoxide Radical scavenging activity

This also one of the radical scavenging assays in which liberated radicals was scavenged by inhibition of formazan formation. The percentage of inhibition was estimated for different solvent extracts which was shown in the figure 2. The BHT was compared and the results were expressed as % of inhibition. In the presence of antioxidant the inhibition was splendid and it was seen in acetone extract. Hot water extract was next to the acetone and unexpectedly methanol extract was next to the hot water extract. Hence the superoxide radical scavenging compound of *A. alnifolia* leaf is much better dissolving in hot water than methanol.

Figure 2
Superoxide radical scavenging activity of A. alnifolia leaf extract



GC-MS

There are 9 compounds in three different RT and peak areas have been observed in acetone extract of *Acalypha alnifolia*. The RT, compound name, peak area percentage and chemical formula were shown in the table 5. The peak height indicates the concentration of the compound in the plant.

Figure 3
Peaks of A. alnifolia leaf acetone extract obtained in GC-MS

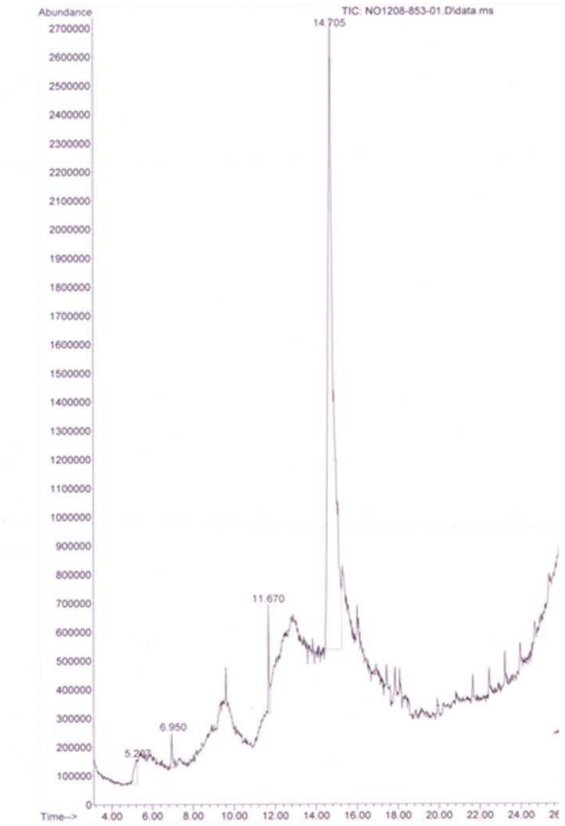


Table 5
GC-MS Compounds with their uses

S.No	RT	Compound	Peak area %	Chemical formula	Reported uses
01	5.207	Cyanoacetylurea	2.27	$C_4H_5N_3O_2$	Used as the pharmaceutical intermediate
02		4-(2-Methylamino) ethyl pyridine		$C_8H_{12}N_2$	It is an antivertigo drug, and for the treatment of atypical depression, obesity management
03		I- Alanine, N-(1-oxopoenyl)-, methyl ester		$C_9H_{17}NO_3$	--
04	11.670	3,5-Dimethyl-1-dimethylphenylsilyl oxybenzene	2.64	$C_{23}H_{26}O_2Si$	--
05		Phenol, 4,4'-methylenebis(2,6-dimethyl)		$C_{17}H_{20}O_2$	Used in fuel, polymers, and lubricant blending industries; used as an antioxidant additive in petroleum-based lubricants
06		Ethanone, 1-[4-methoxy -3-(4-methyl phenoxy) phenyl		$C_{15}H_{13}NO_5$	--
07	14.705	Thiophene, tetrahydro-2-methyl-	94.07	$C_5H_{10}S$	--
08		Myo-Inositol, 4-C-methyl		$C_{19}H_{37}N_5O_7$	--
09		alpha-D- Xylofuranoside, methyl-O-methyl		$C_6H_{12}O_5$	--

DISCUSSION

Search for new antioxidants remains a highly active research area because the antioxidants play a very important role in reducing the risk of various chronic disorders[27]. Many reports have been available that the antioxidant properties in Euphorbiaceae members are mainly due to the presence of high content of secondary metabolite, flavonoids of different types [28-30]. Hence there is no wonder the plant *A. alnifolia* leaf having good antioxidant property.

Extract yield

Traditional knowledge studies concise, the plant extraction was mostly done with (high polar solvent) water to treat different diseases by the ancient traditional healers. The more yield from a particular solvent shows the plant consist more such type of compounds which dissolves more. Hence the leaf of *A. alnifolia* consist more yield while using acetone and methanol.

Quantification of Phenols, Flavonoids and tannins

Total phenol content by Folin Ciocalteau Reagent and *in vitro* antioxidant capacity assays, such as the DPPH, ABTS and free radical scavenging assays (which were used in this study), represent convenient methods for the identification of potential sources of antioxidant compounds[31]. Natural antioxidants mainly come from plants in the form of phenolic compounds (flavonoids, phenolic acids and alcohols, stilbenes, tocopherols, tocotrienols) ascorbic acid and carotenoids. Phenolics having the potential of antioxidant and it can be act against cancer, diabetic retinopathy and chronic inflammation. It inhibits the oxidation of lipids, fats, and proteins (RH) by donation of a phenolic hydrogen atom to the free radical[32]. Phenolic compounds are known as powerful chain breaking antioxidants[33]. It is deduced the leaf extract of *A. alnifolia* may have the potency against above disorders, In addition to that higher the quantity of phenols predicts the higher antioxidant property. Because, in the case of phenolic compounds, the ability of the phenolics to act as antioxidants depends on the redox properties of their phenolic hydroxyl groups, that allow them to act as reducing agents, hydrogen-donating antioxidants, oxygen quenchers[34] . Gulcin *et al.,* reported excellent correlation for medicinal plants when antioxidant activity was compared with total polyphenolic content[35]. Many herbal and plant infusions frequently used in folk medicine have antioxidative and pharmacological properties connected with the presence of phenolic compounds, especially flavonoids[36]. Non-enzymatic antioxidants like phenols, flavonoids and tannins act by one or more of the mechanisms like reducing activity, free radical-scavenging, potential complexing of pro-oxidant metals and quenching of singlet oxygen[2]. Ancient literature regarding antimalarial, antipyretic and laxative property of plant extracts were cited in which quantification of phenols and flavonoids along with tannins[37]. Tannins are known to enhance the synthesis of nitric oxide and relax vascular segments precontracted with norepinephrine acts against cardiovascular disorders[38]. In quantification of acetone and methanol leaf extracts of *A. alnifolia* recorded more phenols and ¾ th of the phenolic compounds consist of tannins. Therefore, the tannins imprint more contribution while pharmacological studies than flavonoids and it will be a good soldier against various metabolic complications.

Antioxidant ability

Many synthetic antioxidant components have shown toxic and/or mutagenic effects, which have shifted the attention towards the naturally occurring antioxidants[39] .The studied medicinal plants revealed interesting antioxidant properties, nutrients and phytochemicals such as phenolics, flavonoids, vitamins, carotenoids, sugars, and fatty acids that could provide scientific evidence for some folk uses in the treatment of diseases related to the production of ROS and oxidative stress[40]. The *A. alnifolia* leaf extract recorded as with good phenolic compounds hence there is no doubt it should

be a good antioxidant capability. Many Synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) commonly used for comparison at the time of searching new antioxidant. Though BHT and BHA are used in processed foods they have side effects and are carcinogenic[41&42]. Hence forth incisive efforts on plants have been undertaken for finding better antioxidant without side effects.

DPPH radical scavenging activity

DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate the antioxidant activity of foods. It has also been used to quantify antioxidants in complex biological systems in recent years[43]. Antioxidant on interaction with DPPH, transfer electron or hydrogen atom to DPPH and thus neutralizing its free radical character and convert it to 1-1 diphenyl-2-picryl hydrazine and the degree of discoloration indicates the scavenging activity of the drug[44]. In the presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases[45]. This interaction depends on the structural conformation of the bioactive compounds present in the plant extracts, among which the hydroxyl groups of flavonoids are highly favourable[46].There was wonderful DPPH radical scavenging potency for not as much quantity of acetone extract. Several flavonoids and polyphenols have been isolated from plant extracts with potent DPPH scavenging activities[47]. Coincidentally, acetone extract contains significant total phenols, flavonoids and tannins than other solvent extracts which enough to predict the potency.

Superoxide radical scavenging activity

Although superoxide anion is a weak oxidant, it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress[48]. Numerous biological reactions generate superoxide anions which are highly toxic species[17]. Superoxide anion radical scavenging activity depends on the redox potential on the actual state of oxidation of the quinones, and oxidized quinones possessed superoxide anion radical scavenging activity[49]. In this present study, though there was no concerning evidence for the presence of alkaloids the acetone leaf extract potentiates to inhibit much better.

ABTS radical scavenging activity

The $ABTS^{\cdot+}$ formed from the reaction in which $ABTS^{\cdot+}$ reacts quickly with ethanol/hydrogen donors to form colourless ABTS radicals. The reaction is pH - independent. A decrease of the $ABTS^{\cdot+}$ concentration is linearly dependent on the antioxidant concentration[17]. In this way the minimum concentration of trolox equal to gram of acetone extract of *A. alnifolia* plant. Though the free radicals may also be a contributory factor in the funcion of the immune system[50], reactive oxygen species are constantly produced during normal aerobic metabolism and are safely removed by a variety of biological antioxidants. Antioxidant protection is never 100% efficient; thus, mechanisms of repair are of key importance for survival[51]. The various methods used to measure antioxidant activity, plant extracts can give varying results depending on the specific free radical being used as a reactant[43]. The presence of different antioxidant components in the plant tissues makes it relatively hard to quantify each antioxidant component separately. Therefore, in many studies, several intermediate extractions are used to ensure a maximum extraction of the available antioxidants[52]. In this view point, the present study is a stone to build alternative antioxidant from *A. alnifolia*.

Phosphomolybdenum reducing power

Collectively, the phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, α-tocopherol, and carotenoids[26]. It is also one of the methods to measure the reducing capability and in some studies it is also known as total antioxidant power assay. Hence the plant *A. alnifolia* methanol extract having potency which mimics the synthetic antioxidants was proved by their total antioxidant capacity.

FRAP

The relationship between phytochemicals and antioxidants have been accessing for predicting antioxidant property of the plants, the presences of abundant total phenolics in several medicinal plants exhibited high FRAP value[53]. Consequently, the leaf extract of *A. alnifolia* results shows exemplary reducing power. So far in addition to that, methanol as a solvent has priority for extraction of plants for evaluating their antioxidant activity[17]. Hence, the acetone and methanol extract of this plant were good hydrogen donating antioxidants.

GC-MS

Phytochemicals are naturally occurring bioactive substances, with various pharmacological action and therapeutic application[54].Several phytochemical screening studies have been carried out in different parts of the world using GC-MS[55]. In addition to this, the results of the GC-MS profile can be used as pharmacognostical tool for the identification of the plant[56]. As per this view, the compound cyanoacetylurea has been used as the pharmaceutical intermediate in various drugs and it appears as white to ivory crystal. Another important compound is 4-(2-Methylamino) ethyl pyridine, commercially known as Betahistidine which was an antivertigo drug, a new use for betahistidine is in the field of obesity management and for the treatment of atypical depression [http://clinicaltrials.gov].

CONCLUSION

As *A. alnifolia* is a wild leafy vegetable plant and its status is nearly endemic, the exploring medicinal property and conservation is necessary. Antioxidants may be actively reducing the free radicals by chain breaking reaction, reducing the concentration, scavenging the initiation, or chelating the metal catalyst. The Plant *A. alnifolia* extracts having any one of the above potency separately or combines with other potency and ultimately it shows the good antioxidant activity. This study solidly concludes the plant *A. alnifolia* having significant antioxidant property which is coincidentally comparable with quantity of phenolics, hence it should be with some pharmacological importance. From this study, searching antioxidant compounds from wild leafy vegetable and making our conventional food as far as possible is a good way to avoid health complication. Exploring the pharmacological properties from conventional food is a supportive tool to aware of traditional medicine importance and the new approach. In this study GC-MS of acetone extract delivering different compounds and some of them reported as with medicinal properties. Hence, further studies on pharmacological properties of *A. alnifolia* in animal model and compound isolation are in prospect.

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