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Evaluation of antioxidant activities by use of various extracts from abutilon pannosum and grewia tenax leaves in the kachchh region

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Abstract

Abutilon pannosum (AP) and Grewia tenax (GT) is a significant medicinal plant widely used in the kachchh region against several diseases. This study was devoted to the determination of antioxidant activity of A. pannosum and G. tenax leaf. Determination of antioxidant activity (AOA) after their successive soxhlet extraction using various solvents with different polarities like n-hexane, benzene, chloroform, acetone, ethyl acetate, acetonitrile and ethanol and also petroleum ether, isopropanol, methanol and water. In the present study following findings were observed: (i) Determination of the AOA in different solvent leaf extracts (DSE) of AP and GT by 2, 2-diphenyl-1- picrylhydrazyl (DPPH), total antioxidant capacity (TAC) by the phosphomolybdenum method (PM) and 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulphonate) (ABTS) method. Among the tested polar solvents like acetone, ethanol, ethyl acetate, acetonitrile and methanol have higher scavenging activity against free radicalsThe data obtained in the present study suggests that the different solvent extract and its methanolic fraction of AP and GT leaves have potent antioxidant activity against free radicals, prevent oxidative damage to major biomolecules and afford significant protection against oxidative damage in the liver.

Keywords: Abutilon pannosum, Grewia tenax, DPPH, TAC (PM), ABTS,

1. Introduction

Medicinal plants that have a significant amount of phytochemicals like phenolic compounds, polyphenols, flavonoids and alkaloids have been described to have multiple biological effects, including antioxidant activity. [1] In modern times, there has been an increase in the use of medicinal plants for therapeutic antioxidant agents. [2] An antioxidant may be defined as 'any substance that when present at low concentrations, compared with those of the oxidizable substrate, significantly delays or inhibits oxidation of that substrate, but later defined them as "any substance that delays, prevents or removes oxidative damage to a target molecule" or Antioxidant is a molecule that inhibits the oxidation of other molecules. [3] Natural antioxidants are known to exhibit a wide range of biological effects including antibacterial, antiviral, anticancer, anti-inflammatory, antiallergic, antithrombic and vasodilatory activities. Antioxidant activity gives rise to anticarcinogenicity, antiimmunogenicity and antiaging activity. [4, 5] Antioxidants are responsible for the defence mechanisms of the organism against the pathologies associated with the attack of free radicals. Thus, the intake of plant derived antioxidant is involved in the prevention of degenerative diseases caused oxidative stress, such as cancer, Parkinson, alzehemir and atherosclerosis. [6] Scientific evidence suggests that antioxidants reduce the risk for chronic diseases, including cancer and heart disease. [7]

Current studies have revealed that there is no worldwide method to evaluate the antioxidant activity quantitatively and precisely, [8] therefore, the AOA of plants is assessed using numerous procedures. These procedures differ in terms of their assay principle and investigational situations. Utmost of them are based on the study of a reaction in which a free radical is produced and how this reaction is repressed by the accumulation of the compound or sample which is the object of the measurement of antioxidant capacity. [9] The effect of the antioxidant activity of the samples differs rendering of the nature of the solvent used and predominantly to the methods of investigation. [10] The presence of different antioxidant components in the plant sample makes it relatively hard to quantify each antioxidant component

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separately. Therefore, in many studies, several intermediate extractions are used to ensure a maximum extraction of the available antioxidants. [11]
In present work anti-oxidant activity of two medicinal plant A. pannosum and G. tenax leaf extracts were evaluated with use of different solvent according to their polarity (like n-hexane, benzene, chloroform, acetone, ethyl acetate, acetonitrile and ethanol and also petroleum ether, isopropanol, methanol and water respectively) by using DPPH, TAC and ABTS. These methods are illustrious by their mechanism of action and would be corresponding to the work of the antioxidant potential of plants. In addition, all extracts were further fractionated by using methanol with reflux through the condenser. After that methanolic fractions were again used for AOA by use of two methods (DPPH and TAC) for evaluation of plant sample activity. Methanol solvent was used in the partition and separation of the various metabolites of different polarity solvents extract as per their solubility. The prospective antioxidant activity of fractions and also different extract which exhibited good antioxidant activity that fraction and extract was also being done using different concentration assays and compared with known antioxidants (Ascorbic acid).

2. Materials and methods

2.1 Antioxidant activity

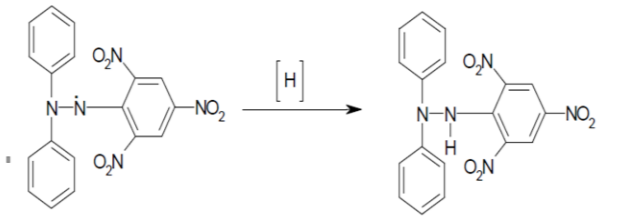
In the present work antioxidant activity of A. pannosum and G. tenax leaf extracts evaluated after successive soxhlet extraction by mainly three methods DPPH, ABTS, TAC (phosphomolybdenum method). Successive extraction was a well technique to extract the antioxidants from medicinal plants than other techniques. [12].

2.1.1 DPPH Method

DPPH is stable free radical at room temperature, the reduction capability of DPPH radical is determined by the decrease in its absorbance at 5l7 nm, induced by antioxidants. The decrease in absorbance of DPPH radical is caused by antioxidants, since of the reaction between antioxidant molecules and radicals, improvements, which results in the scavenging of the radical by hydrogen donation. It is visually perceptible as a change in color from purple to yellow. Hence, DPPH is generally used as a substrate to evaluate the AOA. [13, 14].

2.1.1.1 Principle

The free radical scavenging activity of the extract was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH. Determination of DPPH radical scavenging activity was estimated by the method used by (Kato et al. 1988). **[15]** It offers an accurate and convenient method for determining antioxidant capacity due to the relatively short time required for analysis. The methanolic solution of DPPH is a stable radical which shows peak absorbance at 518 nm. The absorbance disappears due to the reduction of 2, 2'-diphenyl-1-picrylhydrazyl radical (purple color solution) to 2, 2'-diphenyl-1-picrylhydrazine (yellow color solution) [16].



Diphenylpicrylhydrazyl (free radical) *Diphenylpicrylhydrazine (nonradical)*

Figure 1 Reduction of free radical by DPPH

2.1.1.2 Procedure

1mM solution of DPPH in ethanol and also 1mg/1 ml extract solution in ethanol was prepared and 1.5ml of this solution was added to 1.5 ml of DPPH. The absorbance was measured at 517 m against the corresponding blank solution which is prepared by taking 3ml ethanol and control O.D. was prepared by taking 3ml of DPPH. The assay was achieved in triplicates. Ratio inhibition of free radical DPPH was calculated based on control reading by the succeeding equation.

$$\text{DPPH scavenge (\%)} = \frac{(A_{control} - A_{test})}{A_{control}} \times 100$$

Where,
A con - is the absorbance of the control reaction
A test - is the absorbance in the presence of the sample of the extracts [17-20].

2.1.2 ABTS Method

The Antioxidant activity of the samples was measured by ABTS (2, 2-azino-bis (3-ethylbenzthiazoline- 6-sulfonic acid) radical cation decolourization assay according to the method of Re et al, (1999). [21]

2.1.2.1 Principle

ABTS radical scavenging assay comprises a process that generates a blue/green/blues green ABTS+ chromophore through the reaction of ABTS and potassium persulfate. The ABTS radical cation is produced by the oxidation of ABTS with potassium persulfate, reduction capability of ABTS radical is determined by the decrease in its absorbance since hydrogen donating at 734nm, induced by antioxidants. It is visible as a change in color from dark bluish green to colorless. [22].

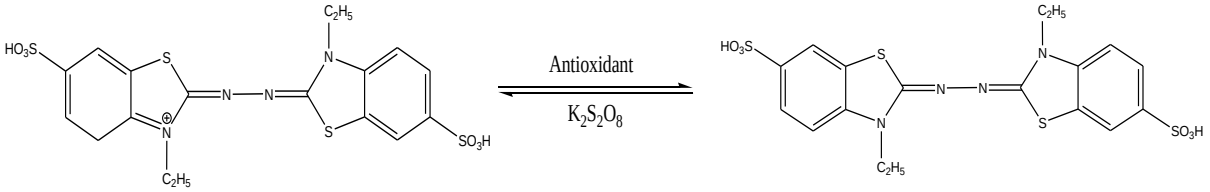


Figure 2: Depicts the formation of ABTS+. As a result of reaction between ABTS and potassium persulfate and the scavenging of the radical cation so formed using ABTS+. Scavenging assay

2.1.2.2 Procedure

ABTS•+ was formed by reacting 7 mM ABTS aqueous solution with 2.4 mM Potassium Per sulphate in the dark situation for 12-16 hour at room temperature. Previous to assay this solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 30^0C to give an Absorbance at 734 nm of 0.700 ± 0.02. Later the addition of 1 ml of diluted ABTS solution to 10 µl of test sample in ethanol absorbance was measured at 30^0C accurately 30 min. after the early mixing. The inhibition percentage was calculated for the blank absorbance at 734 nm. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated via the following formula.

ABTS scavenged (%) = $\frac{(A_{control} - A_{test})}{A_{control}}$ x 100

Where,
A control - is the absorbance of the control reaction
A test - is the absorbance in the presence of the sample of the extracts

2.1.2.3 Preparation of 7 mM ABTS solution

Dissolve 8 mg ABTS in 1 ml water (Solution A). Dissolve 13.2 mg Potassium Per sulphate in 10 ml water (Solution B). Mix 0.5 ml solution A and 0.5 ml solution B above and allow to stand in Dark at room temperature for about 12-16 hour before use. The concentration of ABTS and Potassium Per sulphate are in the mixture and 7 mM and 2.45 mM respectively. The ABTS radical cation in this form is stable for at least 12 -16 hours.

2.1.3 Total Antioxidant Activity

The propensity of plant extract and fractions to diminish molybdate ion was determined according to the technique given by Prieto *et al*. (1999). The phosphomolybdenum method is routinely applied in the laboratory to evaluate the total antioxidant capacity of plant extracts. [23].

2.1.3.1 Principle

The antioxidant capacity of the sample was measured spectrophotometrically through phosphomolybdenum method, based on the reduction of Mo (VI) to Mo (V) by the test sample and the subsequent formation of bluish green phosphate/Mo (V) compounds with a maximum absorption at 695 NM. The phosphomolybdenum method is quantitative one to determine the antioxidant activity in terms of reduction of molybdate ions. The antioxidant activity is expressed in terms of ascorbic acid equivalents as ascorbic acid is used to plot a standard curve [24].

$$M_o^{+6} + A \rightarrow M_o^{+5} + Aoxidized$$

$$M_o^{+5} + PO_4^{3-} \rightarrow \text{Mo3 (PO4)5}$$

Green color complex (λmax = 695nm)

Figure 3: Mechanism of action involved in molybdate reduction assay

2.1.3.2 Procedure

The sample 0.3 ml at different concentration was mixed with 3mL of reagent solution 0.6 M H2SO4, 28 mM sodium phosphate and 4mM ammonium molybdate. The tubes were covered and incubated in a thermal block at 95°C for 90 min. Once cooling at room temperature (28°C), the absorbance of the aqueous solution of all was measured at 695 nm against a blank. Ascorbic acid at different concentration was used as the standard and the total antioxidant capacity is stated as equivalent of ascorbic acid.

2.1.3.3 Preparation of reagent solution

2.1.3.3.1 28 mM sodium phosphate

It was ready by dissolving 3.35 g of sodium phosphate in 1 L of distilled water.

2.1.3.3.2 4 mM ammonium molybdate

4.94 g of ammonium molybdate was dissolved in 1 L of distilled water.

2.1.3.3.3 0.6 M of sulphuric acid

33.33 ml of concentrated (18 N) sulphuric acid was added to distilled water to make up the final volume of the reagent to 1 L. The ascorbic acid was taken as standard and standard curve was obtained using 300-1000µg/ml concentrations.
TAC reagent → Mix (A + B + C) solution equal volume

to make a reagent solution.
The regression equation obtained for ascorbic acid was y = 0.2175x + 0.1476 (R^2 = 0.9965); Here, y = absorbance obtained at 695nm and x = concentration of ascorbic acid used. The reduction ability or antioxidant activity of extracts and different fractions was stated in terms of mg Ascorbic Acid Equivalents (AAE) / 100mg dry weight of extract or fractions as calculated from the standard curve found for ascorbic acid.

3. Result and Discussion

In the present investigation antioxidant activities of different solvent extracts (DSE) and its methanolic fractions (MFE) of *A. pannosum* and *G. tenax* leaf extracts were studied. Different polarity extracts like n-hexane, benzene, chloroform, acetone, ethyl acetate, acetonitrile and ethanol and also petroleum ether, isopropanol, methanol and water and its methanolic fractions of two plants leaves of AP and GT were subjected to antioxidant screening against the DPPH radical, ABTS radical cation and TAC (phosphomolybdenum), because this assays have been widely used to determine the free radical-scavenging activity of various pure compounds or extracts. The ability of different solvent extraction and their methanolic fraction to reduce free radicals was measured by using UV-VIS spectrophotometry. Different solvents dissolve different bio compounds due to differences in their polarity. The factors affecting the choice of solvent are; quantity of phytochemicals to be extracted, the rate of extraction, the diversity of different compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process and potential health hazard of the extract. [25]
Antioxidant capacity calculates might be generally confidential as single electron transfer (SET) and hydrogen atom transfer (HAT) based assays. Preponderances of HAT assays like DPPH and ABTS are kinetics based and include an economical reaction system in which antioxidant and substrate contend for free radicals thermally generated through the disintegration of azo compounds. SET assays measure the capacity of an antioxidant in the reduction of an oxidant which changes colour when reduced. SET assays are easier than HAT assays. SET assays like Phosphomolybdenum (PM) were certain to analyse the reduced capacity. The antioxidant activity of phenolic is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. [26]
When an antioxidant scavenges the free radicals by hydrogen donation (HAT), the color in the DPPH and ABTS assay solutions become lighter and TAC assay involves an electron transfer (SET) mechanism thus solution become color (dark greenish blue). [27]
According to Pérez-Jiménez and Saura-Calixto (2006) the type of solvent and polarity may affect the single electron transfer and the hydrogen atom transfer, which are key aspects in the measurement of antioxidant capacity [28]. In DPPH method there is a loss of colour deep purple to yellow colour due to radical react directly with an antioxidant which absorbs light at 518 nm. [29] In TAC by phosphomolybdenum method there is a affinity of extract and fractions to gain color light yellow to dark greenish blue due to reduced molybdate ions in phosphomolybdenum complex at 695nm. It was stated in terms of number of Ascorbic Acid Equivalents (AAE) in mg / 100mg dry weight of extract or fractions as calculated from the standard curve attained for ascorbic acid [30] and in ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline-6- sulphonate)] radical cation ($ABTS^{\bullet+}$) method there is a reducing of dark blue colour due to oxidation by peroxyl radicals or other oxidants originates the radical cation $ABTS^{\bullet+}$ thus, they can act as reducing agents and hydrogen donators at 734nm. ABTS also known as TEAC (Trolox Equivalent Antioxidant Capacity) assay. [31] Wang et al. found that some compounds which have ABTS+ scavenging activity did not show DPPH scavenging activity. [32] Antioxidant activity depends on the number and position of the hydroxyl groups on the aromatic ring binding site and the type of substitute. Regarding to results, the capacity of the polyphenols to act as antioxidants not only depends on the redox properties of their phenolic hydroxyl groups and the potential for electron delocalization across the chemical structure, as also of the possible reactivity of the reaction products formed during the reaction with DPPH, phosphomolybdenum and ABTS. For this reason, some authors consider that this contribution of the reaction products to the radical scavenging activity limits those methods to evaluate structure-activity relationship as well as correlate the results with the antioxidant activity obtained by other methods. [33] The antioxidant activities correlated with the concentration, chemical structures, and polymerization degrees of sample. [34]
In fact ABTS, TAC and DPPH methods used in the present work, it is very tough to describe the results gained in three methods, hence there is no correlation of activities between the three methods. The antioxidant activity of standards was measured using a spectrophotometric method based on UV-Vis absorption spectroscopy techniques. The graph was plotted against concentration *vs* absorbance at different wavelength according to the method, resulting in a linear relationship as shown in figure 4, 5, 6. Linear regression analysis of DPPH, TAC and ABTS resulted in a correlation coefficient (R2) of 0.9994, 0.9965 and 0.9985 respectively. The resultant standard curve can then be interpolated for determination of antioxidant capacity of unknown samples and reported as their standard equivalent.

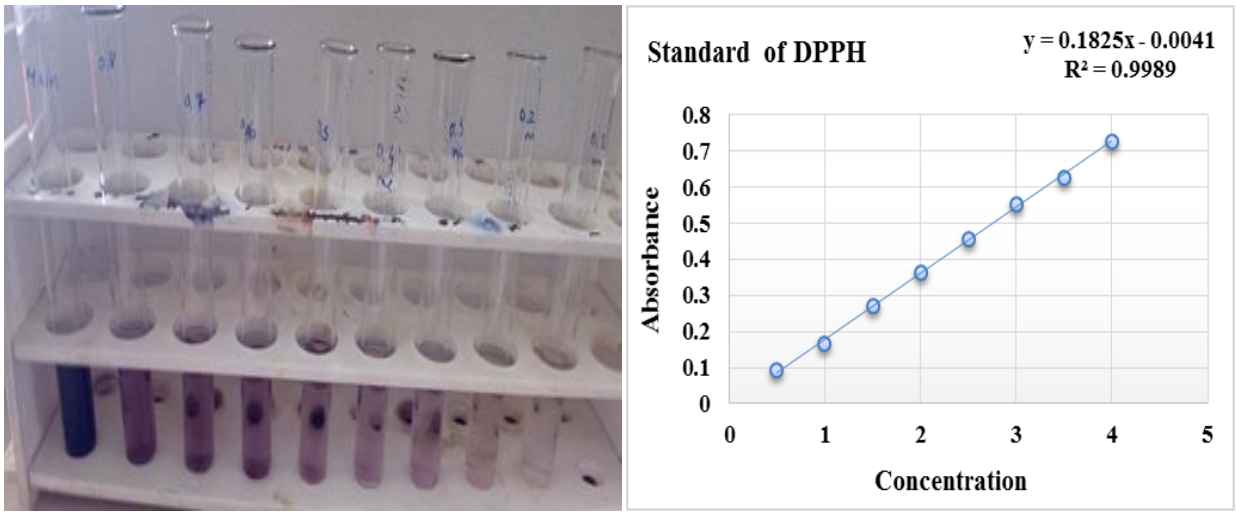


Figure 4 Standard of DPPH

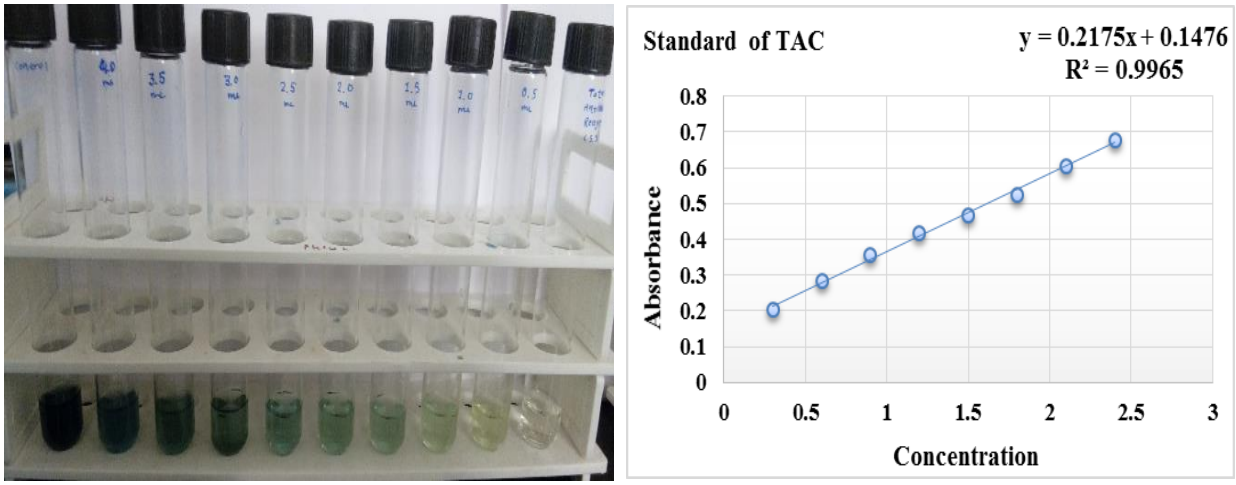


Figure 5 Standard of TAC (Phoshomolybdenum) method

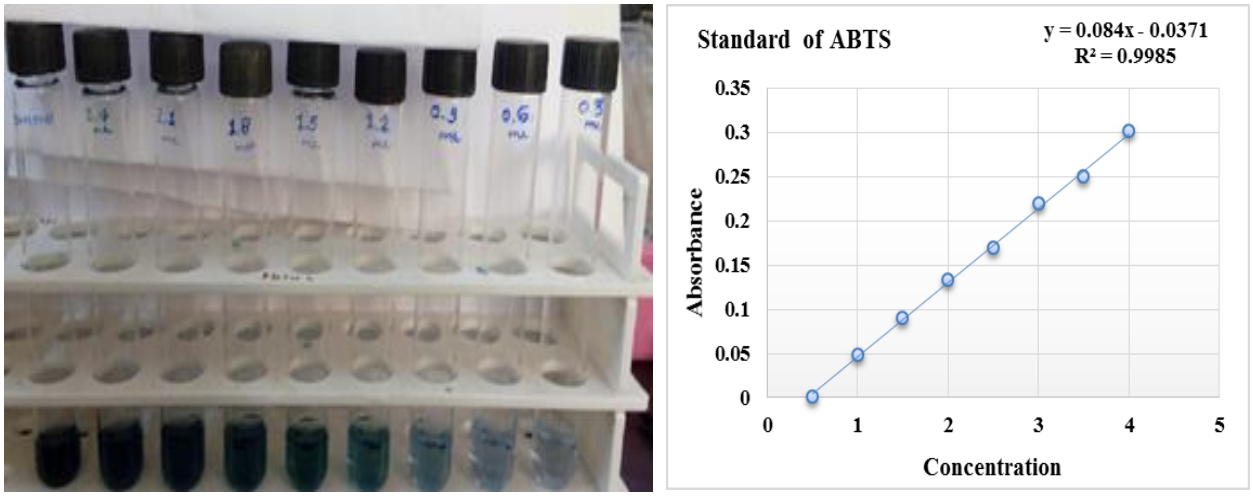


Figure 6 Standard of ABTS method

3.1 Antioxidant activity of A. pannosum and G. tenax plant leaves extract in different solvents by DPPH, TAC (PM) and ABTS method6

3.1.1 DPPH

From the methodological point of view the DPPH• method is recommended as easy and accurate with regard to measuring the antioxidant activity of plant extracts. The results are highly reproducible and comparable to other free radical scavenging methods such as ABTS. [35] DPPH radical scavenging activity increased with increasing phenolic components such as flavonoids, phenolic acids and phenolic diterpenes. These phenolic constituents have several hydroxyl groups, containing an o-dihydroxy group which have very strong radical scavenging effect and antioxidant power. [36] Decrease in absorbance shows the more efficient antioxidant activity of the extract in terms of hydrogen atom donating capacity. The antioxidants present in an extract of leaves of G. tenax and A. pannosum was able to diminish the violet color stable 2, 2-diphenyl-1-picrylhydrazyl radical to the yellow color 2, 2-diphenyl-1-picrylhydrazyl.

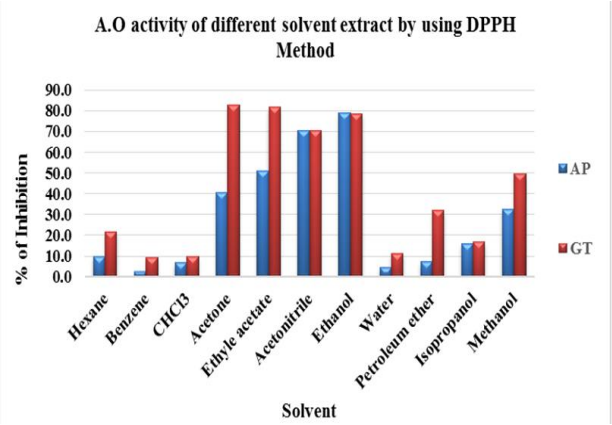


Figure 7 Free Radical Scavenging effect of different solvent extract by DPPH method

This may be due to the neutralization of free radicals (DPPH), either by transfer of hydrogen atoms or by transfer of an electron. [37] The effect of different solvent extract of DPPH radical scavenging activity was observed and shown in Figure 7. In this study, results exhibited that G. tenax has good antioxidant activity compare to A. pannosum. In the assay, free radical scavenging activity was found to be in order of acetone > ethyl acetate > ethanol > acetonitrile extract showed higher activity in G. tenax and ethanol > acetonitrile > ethyl acetate > acetone extract showed strong antioxidant activity in A. pannosum and other solvent contain less amount of scavenging ability. This means phytochemicals soluble in moderate polar solvent possess a stronger potential to scavenge DPPH free radicals. Aqueous plant extracts had not revealed any antioxidant activity with this assay and organic extracts had exposed very stimulating results. The antioxidant proprietors of extracts were measured in terms of their efficient IC 50 concentration consistent to the sample concentration that condensed the initial DPPH• absorbance of 50%. These IC 50 values are given in table 3

3.1.2 Phosphomolybdate (TAC)

The phosphomolybdate method is quantitative, therefore the total antioxidant capacity (TAC) is stated as ascorbic acid equivalent. The capacity of methanol extracts and their fractions to condense molybdate ions was measured by taking ascorbic acid as standard. 0.3-2.4mg/ml concentrations of ascorbic acid were used to attain standard curve and the regression equation gained for ascorbic acid was y = 0.2175x + 0.1476 (R2= 0.9965); Here, y = absorbance found at 695nm and x = concentration of ascorbic acid used. The current study confirmed that *G. tenax* revealed the supreme antioxidant capacity compare to *A. pannosum*. Only petroleum ether and acetonitrile extract of AP exhibited higher A.O activity compare to petroleum ether extract of *G. tenax*. The radical scavenging activity decreased in the following order methanol >acetonitrile > acetone > ethyl acetate > ethanol > petroleum ether > water > benzene > isopropanol > dichloromethane > chloroform in the AP plants leaf extract and methanol > ethanol > acetone > acetonitrile > water > benzene > ethyl acetate > petroleum ether > isopropanol > dichloromethane > n-hexane > chloroform in the GT plant leaf extracts. According to the result in the both plants contain good A.O activity in polar solvent i. e. ethanol, methanol, acetonitrile, acetone etc. Recent work has shown that many flavonoid and related polyphenols contribute expressively to the phosphomolybdate scavenging activity of medicinal plants. [38]

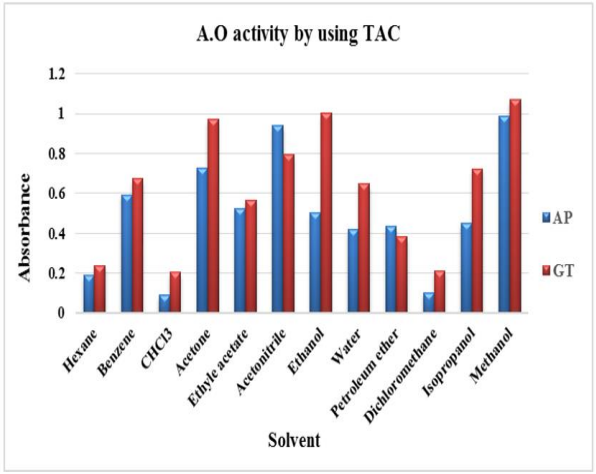


Figure 8 Free radical reducing effect of different solvent extract by TAC method

3.1.3 ABTS Radical scavenging assay

The decolorization of the ABTS•+, through measuring the reduction of the radical cation as the percentage inhibition of absorbance at 734nm (Re *et al.,* 1999). **[21]** ABTS•+ was generated by incubating ABTS•+ chromophore through the reaction. **[36]** The perceived antioxidant of extracts may be due to the neutralization of radical (ABTS), either by transfer of hydrogen atoms or transfer of an electron. **[39]** The radical scavenging activity decreased in the following order ethyl acetate > water > acetonitrile > methanol > petroleum ether > benzene > acetone > ethanol > isopropanol > hexane > chloroform in *A. Pannosum* plants leaf extract and ethanol > acetonitrile > ethyl acetate > methanol > acetone > water > isopropanol > hexane > benzene > petroleum ether > chloroform in the *G. tenax* plant leaf extracts

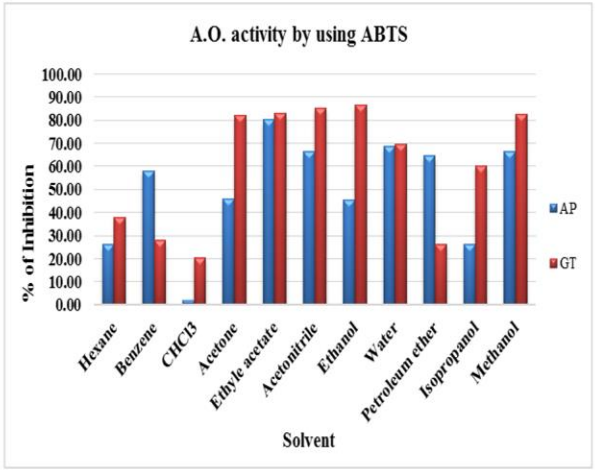


Figure 9 Free Radical Scavenging effect of different solvent extract by ABTS method

Table 1 A.O activity of different solvent leaf extract of A. pannosum and G. tenax by using different types of three method DPPH, TAC and ABTS

Solvent name	DPPH (% SCN)		TAC (Absorbance)		ABTS (% SCN)	
	AP	GT	AP	GT	AP	GT
Hexane	9.83	21.76	0.19	0.238	26.14	52.14
Benzene	2.51	9.31	0.592	0.674	58.14	27.86
CHCl3	7.22	9.83	0.091	0.205	1.86	20.43
Acetone	40.79	82.64	0.725	0.971	46	82
Ethyl acetate	51.15	81.59	0.526	0.568	80.14	83
Acetonitrile	70.19	70.4	0.939	0.794	66.43	85.29
Ethanol	78.66	78.45	0.505	1.001	45.57	86.57
Water	4.81	11.4	0.42	0.648	68.71	69.43
Petro. ether	7.43	31.9	0.434	0.381	64.57	26.29
Isopropanol	16	16.84	0.451	0.723	26.29	60.29
Methanol	32.64	49.69	0.989	1.069	66.29	82.57

3.2 Antioxidant activity of A. pannosum and G. tenax plant leaves methanolic fraction extract of different solvents by DPPH and TAC (PM) method

After studying the free radical scavenging effect of a different polarity solvent extract of both plant leaves by the use of above derive three methods, further all extracted fractioned by using methanol with reflux through the condenser, that methanolic fractions again used of for check scavenging ability of crude extract against free radical. In this assay performed by use of two method DPPH and TAC for evaluated of plant sample activity.

3.2.1 DPPH

The DPPH scavenging activity of different methanolic fractions of leaves of A. pannosum and G. tenax are shown in Figure 10. The ethyl acetate methanolic fraction (EAMF) showed highest DPPH radical scavenging activity in A. Pannosum where as acetone methanolic fraction (AMF) exhibited highest DPPH radical scavenging activity in G. tenax at 1mg/ml concentrations. While water and chloroform showed the lowest amount of radical scavenging activity in AP and GT. The radical scavenging activity in the A. Pannosum

plant leaves extracts decreased in the following order of ethyl acetate (86.30%), acetonitrile (81.38%), ethanol (78.66%), acetone (73.95%), isopropanol (67.36), Petroleum ether (62.66%), n-hexane (61.92%), benzene (51.78%), methanol (32.01%), chloroform (31.80%) and water (0.21%) and G. tenax plant leave extracts decreased in the following order of acetone (90.48%), Ethyle acetate (89.85%), acetonitrile (89.12%), Water (87.76), ethanol (78.45%), Petroleum ether (73.01%), methanol (64.33%), n-hexane (68.10%), benzene (50.73%) and chloroform (42.26%).

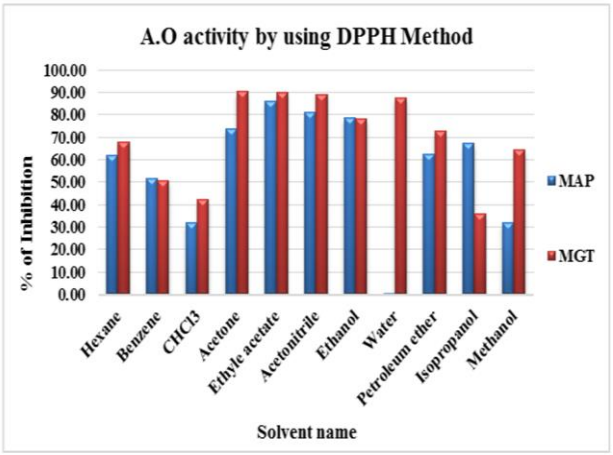


Figure 10 A.o activity of methanolic fraction of different solvent leaves extract of A. pannosum and G. tenax

3.2.2 *Total antioxidant capacity of the phosphomolybdnum method (PM)*

PM method measures the reduction degree of Mo (VI) to Mo (V). These methods are involved in the mechanism of single electron transfer system. In this system electron from oxidized antioxidant moved to the substrate by inhibiting oxidation of oxidant. PM method is based on the redox antioxidant reaction is to assess the reduced concentration of Phosphate-Mo (VI). PM assay gives an instant result of a great range of separate antioxidants in dose-response way. Higher degree of color creation designates the more reducing power of analyte. It is Simple, reproducible investigation. [29].

PM assay is a quantitative method to consider the reduction reaction rate mid antioxidant, oxidant and molybdenum ligand. It includes in thermally producing auto-oxidation during lengthy incubation period at higher temperature. It gives a direct estimation of reducing the capacity of antioxidant. So it shows individuality among in vitro antioxidant assays. [40] The total antioxidant capacity (TAC) of different methanolic fractions of leaves of A. pannosum and G. tenax are shown in Figure. 11 The methanol extract of both plants has very good reducing power and chloroform extract of both plants showed very poor reducing capacity. A. pannosum in the reducing capacity of samples could be observed as well: methanol > dichloromethane > benzene > acetonitrile > ethanol > hexane > ethyl acetate > petroleum ether > acetone > water > isopropanol > chloroform and G. tenax in the reducing capacity showed as well: methanol > ethanol > acetone > isopropanol > hexane > benzene > dichloromethane > acetonitrile > petroleum ether> ethyl acetate > water > chloroform. The antioxidant activity of extracts is strongly dependent on the solvent due to the different antioxidant potentials of compounds with different polarity. [41, 42] It has been reported that in plant compounds with different polarity and structure are present that dissolve in specific solvents having similar polarity. [43, 44]

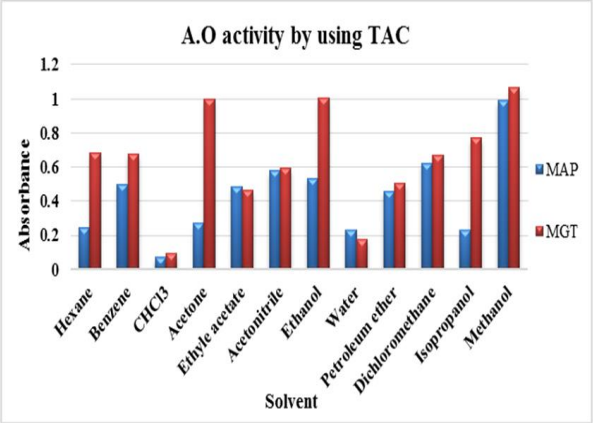


Figure 11 A.o activity of methanolic fraction of different solvent leaves extract of A. pannosum and G. tenax by TAC method

Table 2 Antioxidant activity of A. pannosum and G. tenax plant leaves methanolic fraction extract of different solvents by DPPH and TAC (PM) method

Solvent name	DPPH (% SCN)		TAC (Absorbance)	
	MFAP	MFGT	MFAP	MFGT
Hexane	61.92	68.1	0.245	0.682
Benzene	51.78	50.73	0.501	0.678
CHCl3	31.8	42.26	0.072	0.096
Acetone	73.95	90.48	0.27	0.999
Ethyl acetate	86.3	89.85	0.485	0.461
Acetonitrile	81.38	89.12	0.578	0.592
Ethanol	78.66	78.45	0.535	1.003
Water	0.21	87.76	0.23	0.177
Petroleum ether	62.66	73.01	0.46	0.508
Isopropanol	67.36	35.77	0.229	0.771

3.3 Comparison between different solvent extract and its methanolic fraction of A. pannosum and G. tenax plant leaves by DPPH and TAC (PM) method

The antioxidant activity result of the different solvent extracts and its methanolic fraction for two medicinal plants A. pannosum and G. tenax were obtained. In the present assay comparison between DSE and its MFE. In DPPH method MFE of all extract obtained from a different solvent crude extract exhibited higher antioxidant activity comparable to that DSE, Thus, this might be due to the fact that crude of DSE have a tendency to more interfering substances as compared to fractions. [45] While the contrary result showed in TAC (PM), DSE exposed higher adsorption compare to its MFE, except of n-hexane, petroleum ether, dichloromethane. This may due to DSE have more interfering substances so that compound formed complex structure via thermal process. That result solution color appears to become dark green. Thus, in TAC different solvent crude extract showed higher values compared to its methanolic fraction. The result shown above table 1 & 2 and also below figure 12, 13 and 14.

The results of the present investigation demonstrate that a methanol could be increases of different solvent crude extract efficiency toward scavengingabilityy of free radicals. Only methanol extract existed weak antioxidant activity while the methanol fraction of different crude extract provided stronger antioxidant activity. The results designate that methanolic fractions found from different solvent crude extract display higher antioxidant activities as associated to the only methanol solvent extract. This could be due to the fact that crude (methanol) extract tends to have more interfering substances as compared to fractions. [46] The results obtained of TAC that a DCE were shown higher absorption compare that of MFE but only methanol extract existed a very higher absorption capacity compare to other solvent extract in both plants. This may due to methanol consist hydroxyl group which is mainly responsible for the increase antioxidant capacity. [47]

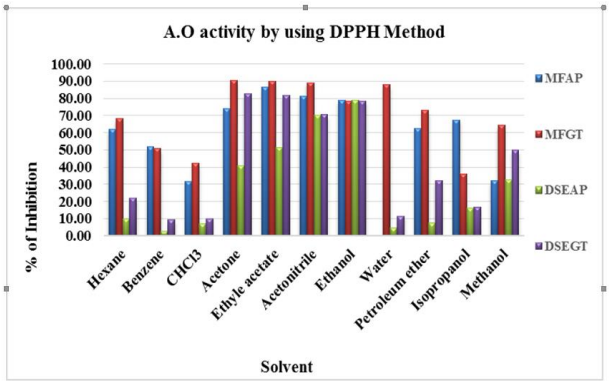


Figure 12 Comparison between diifferent solvent extract and its methanolic fraction by DPPH method [DSEAP=Different solvent extract of A.pannosum, DSEGT=Diifferent solvent extract of G. tenax, MFAP= Methanolic fraction of A.pannosum, MFGT=Methanolic fraction of G. tenax

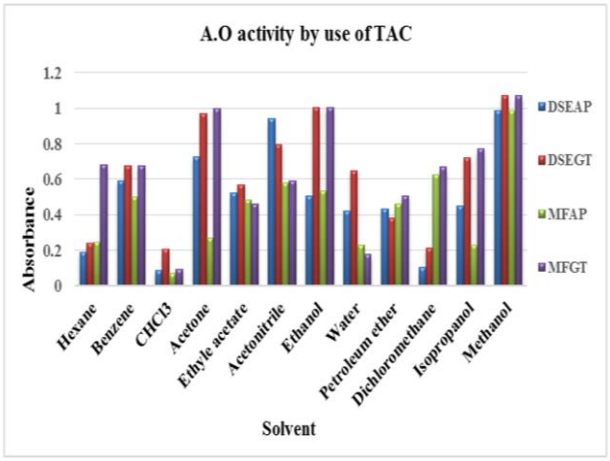


Figure 13 Comparison between different solvent extract and its methanolic fraction by TAC method

3.4 IC50 of DPPH scavenging activity of A. pannosum and G. tenax leaf different solvent extract and its methanolic fraction which exhibited well AOA

The antioxidant activities of the plant extract were determined on the base of the free radical scavenging influence of stable DPPH free radical. [48] The reaction mixture (3.0 mL) consisting of 1.5 mL DPPH (1mM), 1.5 mL diverse concentrations (30 to 150 µg/ml) of DSE was incubated for 10 min, in the dark, after which the absorbance was measured at 517 nm using a UV–VIS Spectrophotometer (Shimadzu, Japan). The absorbance of each solution was determined, the corresponding blank readings were also taken and remaining DPPH was calculated. Decrease in absorbance illustrations the more competent antioxidant activity of the extract in terms of hydrogen atom donating capacity. This assay is more indirect type as it measures the inhibition of reactive species (free radicals) produced in the reaction mixture and its results depend on the type of reactive species use. [49].

The scavenging activity was stated as IC50 (µg/ml). The IC50 was used to categorize the quality of the antioxidants in the sample extracts that compared to standard. It was perceived that, the DPPH radical scavenging activity of the tested extracts amplified with the quantity of plant material in the extract. IC50 of DPPH scavenging activity is the concentration of sample or standard that can prevent 50% of DPPH scavenging activity. IC50 values are contrariwise proportional to the antioxidant activity of plant extracts. The lowest IC50 means had the highest antioxidant capacity. The sample that has an IC50 fewer than 50 µg/ml is a very strong antioxidant, 50-100 µg/ml is a strong antioxidant, 101-150 µg/ml is a medium antioxidant, whereas IC50 greater than 150 µg/ml is a weak antioxidant. [50] IC50 values were calculated by applying suitable regression analysis of the mean inhibitory values of DPPH radical. Figure 21 shows the concentration dependent response curve of

DPPH scavenging activities of the different solvent crude extract and methanolic fractions of leaves of AP and GT. The DPPH solution without sample solution was used as a control. IC50 values is the concentration of a sample (mg/ml) required to scavenge 50% DPPH free radical and was calculated from inhibition curve. [51].

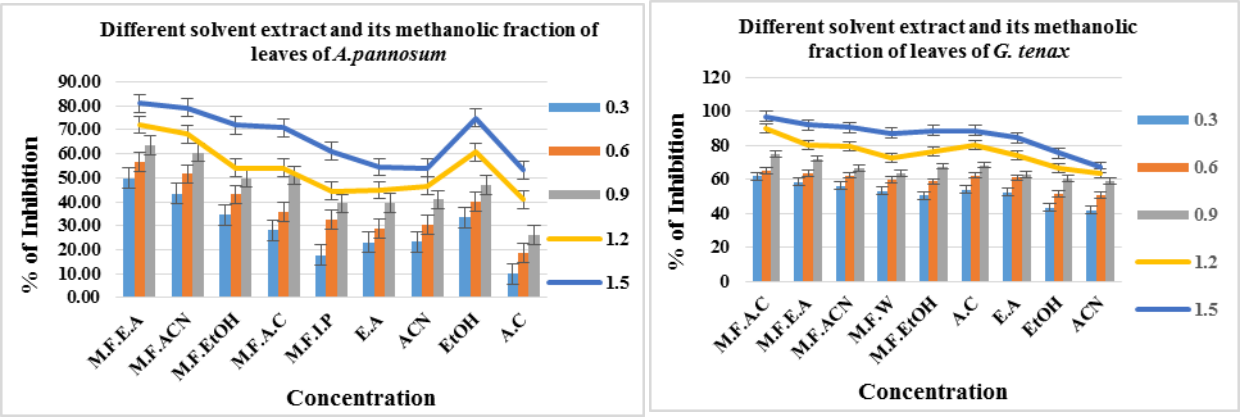


Figure 14 % of Inhibition of different conc. of plant extracts for free radical scavenging activity by DPPH radical. Extracts: M.F.E.A = Methanolic fraction of ethyl acetate, M.F.ACN = Methanolic fraction of acetonitrile, M.F.EtOH = Methanolic fraction of ethanol, M.F.A.C = Methanolic fraction of acetone, M.F.I.P = Methanolic fraction of isopropanol, E.A = Ethyl acetate, ACN = Acetonitrile, EtOH = Ethanol, A.C = Acetone

Table 3: IC50 of DPPH scavenging activities in various leaf extracts from two plants of A. pannosum and G. tenax

Sr. No.	Extracts	IC50 - G.T	IC50 - A.P
1	M.F.A.C	0.023	0.959
2	M.F.E.A	0.072	0.338
3	M.F.ACN	0.167	0.541
4	M.F.W/I.P	0.259	1.234
5	M.F.EtOH	0.317	0.739
6	A.C	0.193	1.46
7	E.A	0.242	1.354
8	EtOH	0.541	0.866
9	ACN	0.652	1.323

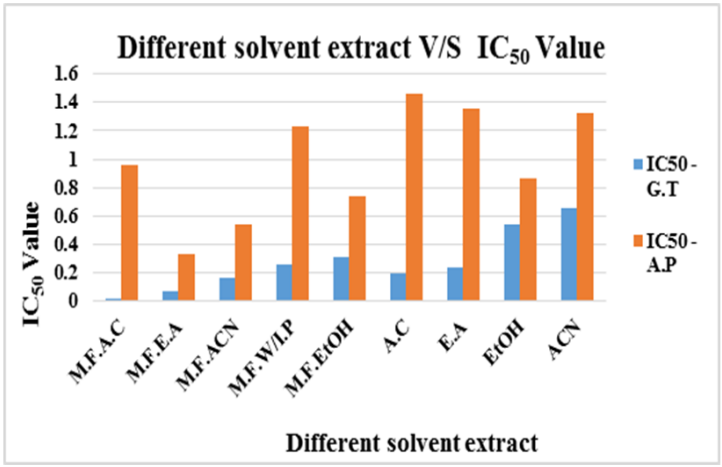


Figure 15 IC50 of DPPH scavenging activities in various leaf extracts from two plant of A. pannosum and G. tenax

The current study revealed that IC50 of DPPH scavenging activities of ethyl acetate (E. A), acetonitrile (ACN) ethanol (EtOH), acetone (A.C), isopropanol (I.P), water (W) and its methanolic fraction (M.F) of leaf extracts of *A. pannosum* and *G. tenax*. In this study, results showed that all samples had significant levels of radical scavenging activity (Fig. 15). According to the results exhibited that antioxidant capacity for all the extracts were found in the range of 0.338 to 1.460 in *A. pannosum* and 0.0232 to 0.652 in *G. tenax* thus, *G. tenax* contained highest antioxidant activity compare to *A. pannosum* and also showed that the methanolic fraction of different solvent extract gives very strong antioxidant activity comparable to that solvent extract fraction. The highest radical scavenging activity was shown by a methanolic acetone extract fraction of *G. tenax* by the low IC50 value of IC50=0.0232 mg ml-1 which is higher than other all extracts and acetone leaves extract of *A. pannosum* showed lowest DPPH radical scavenging activity, as described by high IC50 values of IC50=1.460mg/ml and IC50 values of standard graph 273.995 mg/ml was observed. The radical scavenging activity in the *A. pannosum* and *G. tenax* plant extracts decreased in the following order M.F.E.A > M.F.ACN > M. F. EtOH > EtOH > M.F.A.C > M.F.I.P > ACN > E. A > A.C and M.F.A.C > M.F.E.A > M.F.ACN > ACN > E. A > M.F.W > M. F. EtOH > EtOH > A.C, respectively. All of *A. pannosum* and *G. tenax* leaves extracts with different polarity solvents (Acetone extract, isopropanol extract, ethyl acetate extract, Milli-Q- water extract, acetonitrile extract, ethanol extract and its methanolic fraction extract) can be categorized as very strong and a strong antioxidant. It has well established that free radical scavenging activity of plant extracts is mainly due to flavonoids, carotenoids and phenolic compounds. [52] Further work is essential to find the components in phenolic and flavonoids that may have contributed to the greater antioxidant activity. [53]

3.5 Effect of time on scavenging activity of A. pannosum and G. tenax leaf methanolic fractions obtained from a different solvent crude extract by DPPH method

The time is the most important factor affecting to the compounds antioxidant capacity. [54] Time of reaction also produced diverse results thus, in present part we were focused on the effect of time depend factor on different methanolic fraction extract like ethyl acetate, acetonitrile, ethanol and acetone. In industry, time is a very important factor to be enhanced even in order to diminish energy rate of the process. [55] The effects of time on the antioxidant capacity of APMF and GTMF extract were revealed in Figure 16 to 23. The sample extract was estimated at same time difference 30, 60, 90 and 120 minutes. Generally, 30 minutes of reaction provided lower scavenging activity compare to 120 minutes. That means AOA was increased as the time increased. The maximum AOA was shown, in order of MFEA (89.6 %), MFACN (85.2 %), MFEtOH (76.6 %) and MFAC (70.5 %) was achieved in 120 minutes in AP and MFAC (96.7 %), MFEA (93.9 %), MFACN (90.7 %), and MFEtOH (89.5 %) was attained in 120 minutes in GT. This may be due to the Scavenging ability of sample extract against free radical an increased with increased time as a certain limit. According to the result of AOA none major differences between 90 and 120 minutes. This showed that the prolonged time do not significantly enhance the antioxidant activity of the extracts. This finding was related to the Fick's second law of diffusion, which indicated that the solute concentration in the plant matrix will achieve final equilibrium with the bulk solution over a certain period of time. [56] Furthermore, the extreme course of period will cause degradation of phenolic compounds due to light and oxygen exposure leading to the phenolic oxidation. [57] Which may have responsible for reduce AOA power thus, the time of 90 and 120 minutes were favourable for good result

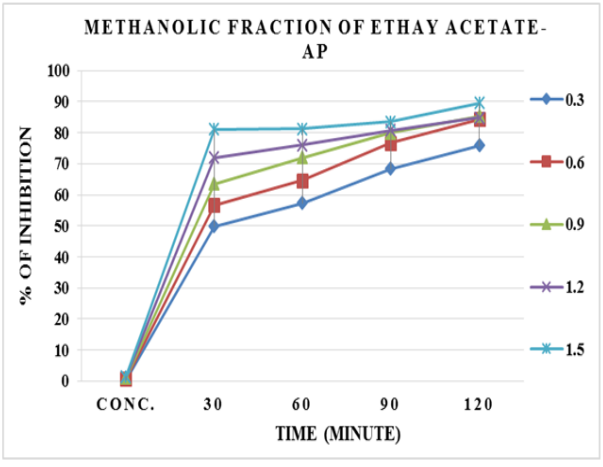


Figure 16 Effect of time on the scavenging of methanolic fraction of Ethyl acetate -AP

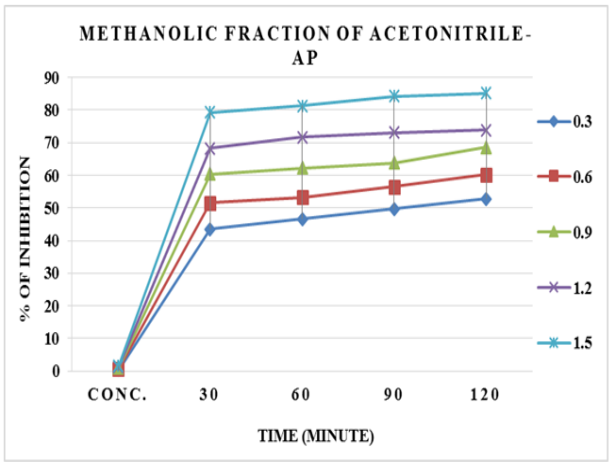


Figure 17 Effect of time on the scavenging of methanolic fraction of Acetonitrile -AP

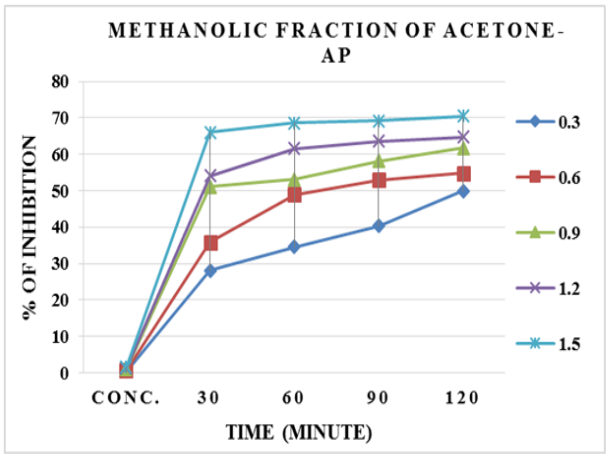


Figure 18 Effect of time on the scavenging of methanolic fraction of Acetone -AP

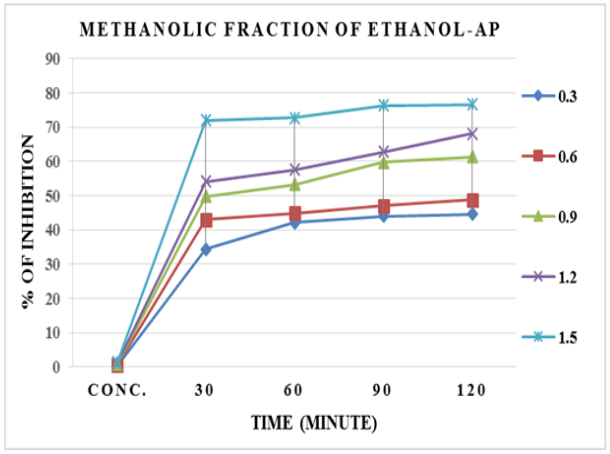


Figure 19 Effect of time on the scavenging of methanolic fraction of Ethanol -AP

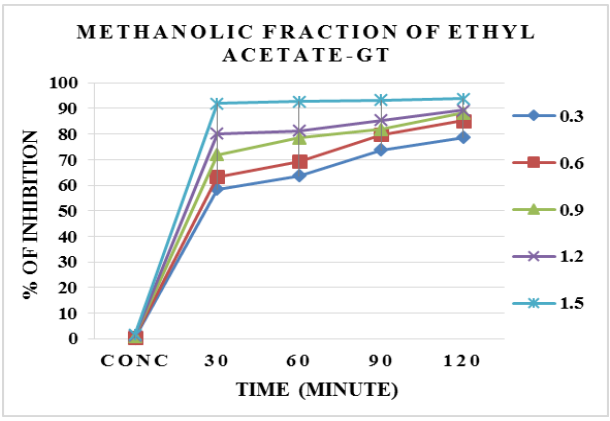


Figure 20 Effect of time on the scavenging of methanolic fraction of Ethyl acetate -GT

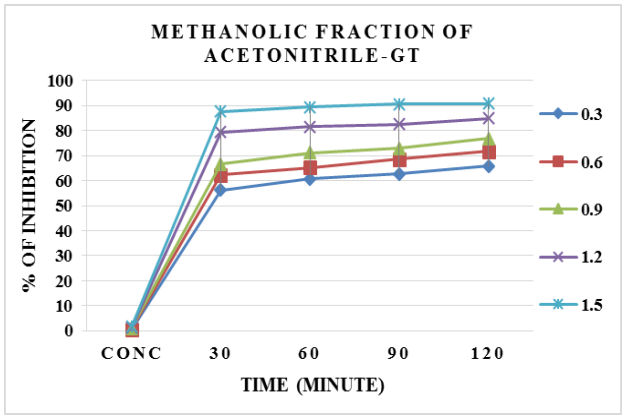


Figure 21 Effect of time on the scavenging of methanolic fraction of Acetonitrile - GT

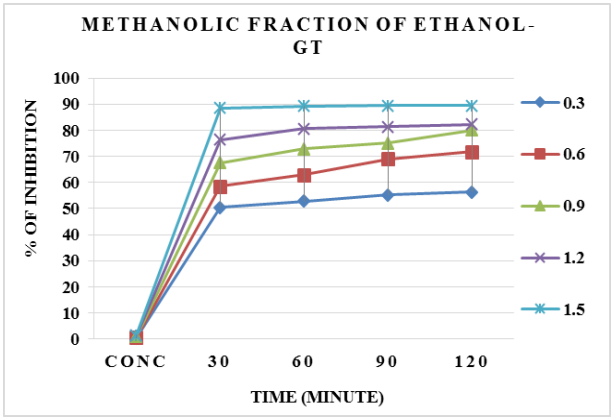


Figure 22 Effect of time on the scavenging of methanolic fraction of Ethanol -GT

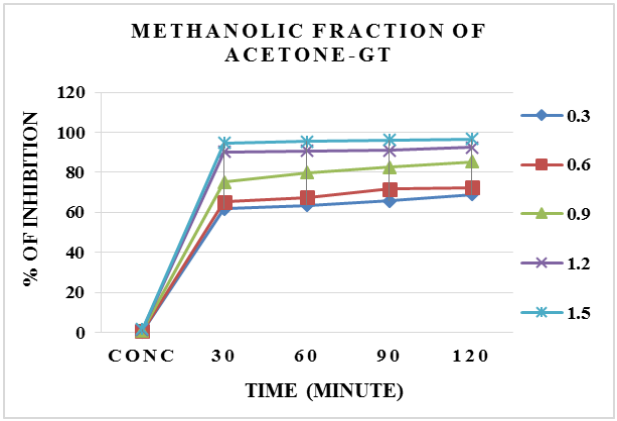


Figure 23 Effect of time on the scavenging of methanolic fraction of Acetone - GT

Table 4 Effect of time on Scavenging of methanolic fraction extracts by use of DPPH Method

Abutilon pannosum																
	MFE of Ethyl acetate				MFE of Acetonitrile				MFE of Ethanol				MFE of Acetone			
Conc.	30	60	90	120	30	60	90	120	30	60	90	120	30	60	90	120
0.3	49.8	57.2	68.4	75.8	43.4	46.5	49.7	52.8	34.4	42.1	44.0	44.6	28.1	34.6	40.3	49.9
0.6	56.7	64.6	76.7	84.3	51.5	53.2	56.5	60.2	43.0	44.9	47.0	48.8	35.8	49.0	52.9	54.8
0.9	63.4	72.0	80.0	85.3	60.4	62.3	63.7	68.5	49.7	53.2	59.8	61.4	51.0	53.1	58.2	61.7
1.2	72.0	76.0	80.7	84.8	68.2	71.7	73.1	73.9	54.0	57.6	62.8	68.1	54.1	61.6	63.6	64.8
1.5	81.1	81.3	83.6	89.6	79.2	81.3	84.3	85.2	72.0	72.7	76.3	76.6	66.1	68.6	69.2	70.5
Grewia tenax																
	MFE of Ethyl acetate				MFE of Acetonitrile				MFE of Ethanol				MFE of Acetone			
Conc.	30	60	90	120	30	60	90	120	30	60	90	120	30	60	90	120
0.3	58.5	63.6	73.9	78.7	56.1	60.7	62.7	65.8	50.5	52.8	55.3	56.3	61.7	63.4	65.7	68.9
0.6	63.3	69.4	79.7	85.2	62.3	65.1	68.6	71.6	58.6	62.9	69.1	71.7	65.1	67.4	71.7	72.3
0.9	72.0	78.6	81.8	88.3	66.5	71.1	72.8	76.9	67.5	72.8	75.1	80.1	75.0	79.9	82.4	85.2
1.2	80.2	81.3	85.4	89.3	79.4	81.6	82.5	84.8	76.3	80.6	81.3	82.2	90.1	90.6	91.0	92.4
1.5	92.0	92.7	93.3	93.9	87.7	89.5	90.5	90.7	88.6	89.4	89.5	89.5	94.7	95.5	96.1	96.7

4. Conclusion

Physiochemical properties of the sample are very important for analyzing antioxidant properties. The purpose of the present study is to explore the antioxidant potential of different solvent extracts and its methanolic fractions of *A. pannosum* and *G. tenax* leaves powder, were made using the successive soxhlet extraction method using various solvents with different polarity. Great levels of antioxidant activity (AOA) were detected in *G. tenax* leaf as compared to *A. pannosum* leaf indicating that the *G. tenax* leaf may serve as an excellent source of natural antioxidants. During comparison between different solvent extract and its methanolic fraction of AP and GT plant leaves by DPPH and TAC (PM) method. In the DPPH assay methanolic fraction of different solvent extract had higher AOA value compare to different solvent extract and TAC assay different solvent extract exposed higher adsorption compare to its methanolic fraction except of n-hexane, petroleum ether and dichloromethane. The % of free radical scavenging increase with increase concentration of extract as well as time. The Concentration expressed as IC_{50} values. IC_{50} values are inversely proportional to the antioxidant activity of plant extracts. As per DPPH assay result % of free radical scavenging increase with decreases IC_{50} values and increase time as certain limit. Therefore, it can be definite that both plant extracts possess strong antioxidant activity. According to the above results we can conclude that the antioxidant capacity of sample extract was dependent on the type of solvent, concentration of the extract, AOA method and also reaction time. The outcomes of this work are useful for further research to identify, isolate and characterize the exact compound which is accountable for greater antioxidant activity.

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