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Research Article

Antioxidant potentials in leaves of *Kigelia, Thespesia, Eucalyptus, Peltophorum* and *Azadirachta.*

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ABSTRACT

Phytochemical is a natural bioactive non reactive compound found in plants that works with nutrients and dietary fibers to protect and prevent diseases. Leaves of selected trees from Gujarat University campus was used to make extracts with solvents namely, methanol and acetone. Phyotchemical screening was performed using standard protocol for all the prepared extracts where alkaloids phenols and tannins were found to be present. In the DPPH Assay, 0.01mM DPPH solution was made and the standard considered was L- Ascorbic Acid. The % inhibition activity in *Eucalyptus,* at 0.5 mg/ml for its methanol extract is 98.17±1.380 while in *Peltophorum,* at 0.5 mg/ml for its acetone extract it is 97.33±0.153 mg/ml. The inhibition activity for *Azadirachta* was found to be lower as compared to that of methanol extract of *Eucalyptus* and Acetone extract of *Peltophorum.* IC_{50} values of *Azadirachta* acetone extract had the highest value and *Eucalyptus* had lowest while, methanol extract of *Thespesia* showed the maximum value and the lowest values were recorded for *Peltophorum* and *Eucalyptus.*

Keywords: Leaves, *Kigelia, Thespesia, Eucalyptus, Peltophorum, Azadirachta,* Acetone extracts, Methanol extracts, Phytochemical Screening, DPPH Assay and IC_{50} Values.

INTRODUCTION

The green protective shield "The Trees" have been a part of the existence and sustainability of all living forms found evolving in the nature. They are beneficial and endless sources for healing various infections because of their ability to synthesize secondary metabolites like alkaloids, quinines, flavones, tannins, phenols, etc. These serve as plant defense mechanisms against microorganisms, insects, and herbivores and also act as natural source for new pharmaceutical preparations [27]. The leaves of the trees are abundantly available and so their potentialities are worth finding as to which extent they can be useful to use. The phytochemicals found in leaves of plants commonly found growing around us are still unknown.

The present study aims to reveal the exceptional properties of the leaves of *Kigelia pinnata* (Jacq) DC., *Thespesia populnea* (L.) Soland. ex. Correa., *Eucalyptus globulus* Lablil., *Peltophorum pterocarpum* (DC.) K. Heyne. and *Azadirachta indica* L. extracts prepared using solvents, methanol and acetone to evaluate their capacity to scavenge free radicals.

MATERIAL AND METHODS

Sample Collection and Extract Preparation

The experimental was conducted in the Ecology laboratory of the Department of Botany, Gujarat University, Ahmedabad (Gujarat). Leaves of the tree species namely *Kigelia*, *Thespesia*, *Eucalyptus*, *Peltophorum* and *Azadirachta* were collected washed with tap water then with distil water. These leaves were then sun dried and powdered. 10gm of leaf powder for each tree species were kept in the 100ml solvent (Acetone and Methanol) for 48 hours. After a process of filteration using Whattman Filter paper No. 1. the filterate obtained was kept at room temperature for evaporation of the selected solvent (acetone and methanol), thus crude extracts were

obtained. Phtyochemical screening was done using the standard protocol [10] later DPPH Assay was done.

DPPH Assay

The DPPH Assay was done using the methanol and acetone extracts of the leaves of five tree species and thus they were tested for their antioxidant activity [9]. The experiment was done in triplicates (n=3).

The concentration series for the extract and Standard (Ascorbic acid-L) ranged from 0.1 mg/ml to 0.5 mg/ml. The concentration of DPPH (2,2-diphenyl-1-picrylhydrazyl) was taken as 0.01mM (4mg DPPH/100 ml Methanol) and the standard considered was Ascorbic Acid –L. The standard graph for Ascorbic acid is given (**Fig. 1**).

The capability to scavenge the DPPH free radical was calculated using the following equation[17]:

$$\% \text{ Radical Scavenging Activity} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100\%$$

(where, Abs control = Absorbance of DPPH solution and Abs sample = Absorbance of extracts and ascorbic acid solutions).

For IC_{50} values calculation: Scatter Graph type was inserted after selecting both columns of % Inhibition for each leaf extract in excel sheet. When clicked on any data point on the graph, and then add trendline (after right click) a linear regression formula would be obtained on the graph. An equation with R^2 value, was based on $Y = mx + C$ (where Y = % Inhibition, x= Concentration, C = Constant and m= Coefficient) Then, simple mathematics can be used to obtain the inhibitory concentration which inhibit 50% free radicals in DPPH.

RESULT and DISCUSSION

Results

Phtyochemical screening was done for both the extracts prepared using acetone and methanol as solvents for extraction. The screening of Secondary metabolites was performed to detect the presence of metabolites namely Alkaloids, Flavonoid, Phenol, Tannins, Saponins, Steroids and Glycosides (**Table 1 and 2**).

The DPPH Assay was done using the methanol and acetone extracts. The extracts of the selected tree species were tested for their antioxidative potential. The concentration for the extract is taken from 0.1 mg/ml to 0.5 mg/ml while 0.01 mM of DPPH was taken.

The % inhibition activity of DPPH Assay for *Eucalyptus* has been found to be high at the concentration of 0.5 mg/ml *i.e* for 92.87±0.208 (for acetone extract) and 98.17±1.380 (for methanol extract) (**Fig. 2**).The % inhibition activity of DPPH Assay for *Peltophorum* has been found to be high at the concentration of 0.5 mg/ml *i.e.* 97.33±0.153 (for acetone extract) and 97.43±1.210 (for methanol extract) (**Fig. 3**).The % inhibition activity of DPPH Assay for *Kigelia* has been found to be high at the concentration of 0.5 mg/ml *i.e.* 85.23±0.252 (for acetone extract) and 96.4±0.265 (for methanol extract) (**Fig. 4**).The % inhibition activity of DPPH Assay for *Thespesia* has been found to be high at concentration of 0.5 mg/ml *i.e.* 77.1±0.265 (for acetone extract) and 96.9±0.265 (for methanol extract) (**Fig. 5**). The % inhibition activity of DPPH Assay for *Azadirachta* has been found to be high at concentration 0.5 mg/ml *i.e.* 82.87±0.208 (for acetone extract) and 95.57±0.252 (for methanol extract) (**Fig. 6**).

A comparative graphical representation above shows the overview of all the selected tree species leaves extract's DPPH Assay with the Standard (Ascorbic acid-L) which is essential for the analysis of the activity of the secondary metabolites that have the innate property to scavenge free radicals (**Fig. 2-6**).

Discussion

Secondary metabolite screening for *Kigelia* showed that Alkaloids, Flavonoids, Phenols, Tannins and Saponins were present in both AE and ME. The results of this experiment were same as that of the findings of [2] [23] [33]. Steroid were absent in both AE and ME which does not support the work done by [2] [23] [33] [30]. Glycosides are present in ME and absent in AE (**Table 2**).

Secondary metabolite screening for *Thespesia* showed Alkaloids, Flavonoids, Phenols, Tannins, Glycosides and Saponins were present in both AE and ME. Steroids were absent in both AE and ME. According to the research done on the phytochemical composition tested are almost the same the only difference is that in the ME and AE extracts both lack Steroids which does not support the earlier research work done by [36] [7] (**Table 2**).

Secondary metabolite screening for *Azadirachta* leaves; Alkaloids are present in both AE and ME. The results were similar to that of [8] [27]. Phenols are present in ME and absent in AE. the results supports[8]. Flavonoids are present in AE. Flavonoids are absent in Alkaline reagent test, Pew test and Shinoda test for ME. Flavonoids are present in Zinc Hydrochloride test and dilute ammonia test for ME. The presence of flavonoids was proved by [8]. These results do not support the work done by [22] whose research showed the absence of Flavonoids in the *Azadirachta* leaves. Saponins are present in ME

while absent in AE, these results are similar to that of [22] [8] [27]. Steroids were absent in both AE and ME. The steroids are absent as no data is available to support its presence while Carbohydrates are present in previously done research work according to [3] while the experimental results show that it was absent. Glycosides are present in ME and absent in AE. Glycosides have not yet been found present in any of the research works but they have been found to be present in the ME. Tannins are absent in AE for Ferric Chloride test and Potassium dichromate Test, while are present in Lead-Acetate test. Tannins are present in all the ME. Tannins are present in the given tree species as the experimental results can be supported to that of [8] (**Table 1**).

Secondary metabolite screening for Peltophorum leaves Alkaloids, Flavonoids, Phenols, Tannins and Saponins are present in both AE and ME. These results were similar to that of [18]. Glycosides are present in ME but absent in AE. There has not been any research on the presence of Glycosides in Peltophorum (**Table 1**).

Secondary metabolite screening for Eucalyptus leaves five of the phytochemicals tested have positive results they are Alkaloids, Flavonoids, Tannins, Saponins and Phenol for both the extracts. These five metabolites have been found to be present in most of the research works done by [5] [28] [34] which support the experimental results. Steroids showed negative results. Glycosides are absent in AE while present in ME, Eucalyptus leaves have Glycosides present in various studies done by [28] [34] that supplements the results obtained (**Table 1**).

The DPPH assay were conducted for the calculation of the % inhibition activity for both extracts of tree's leaves.% Inhibition of Acetone and Methanolic extract of *Eucalyptus* at showed that the inhibitory activity was higher for the Methanolic extract as compared to Acetone extract. The maximum activity was of 98.17% at 0.5 mg/ml conc. for the Methanolic extracts of Eucalyptus (Table 2, Fig. 1) results were similar to [6] [15] [16] [32] . The comparative graph of the two extracts with the standard shows that the Methanolic extract has almost similar inhibitory conc. of that of Standard (**Fig. 2**). The Acetone extract has less inhibitory activity as compared to the Methanolic extract and the Standard. The inhibition of Acetone extract is 5.3% less than the Methanolic and about 6.9% less than Standard at the maximum inhibitory concentration considered for the experiment that coincide with results of [11] [21] [14]. % inhibition of ME is higher than that of AE for leaves extract of *Peltophorum* (**Fig. 3**). The inhibitory activities of both the extracts are almost similar as at 0.5 mg/ml conc. both for AE and ME have only difference of 0.1% in their % Inhibition. The comparative graph of AE, ME and Standard shows that at 0.3 mg/ml conc. the AE of *Peltophorum* has high inhibition as compared to the ME (**Fig. 3**). While for 0.4 mg/ml and 0.5 mg/ml conc. the % inhibition is almost negligible[12] (**Fig. 3**). The ME of *Kigelia* has a significant inhibitory activity than AE. The % inhibition of ME is higher as compared to that of the AE at the maximum conc. considered i.e., of 0.5 mg/ml (**Fig. 4**). There is a vast difference of the inhibitory activity of both the extract i.e., about 11% at 0.5 mg/ml. concentration (**Fig. 4**), such conclusions have also been observed [23] [31] [1] [4]. The comparative graph of AE, ME and Standard has a sequential increase in the inhibition with increasing conc. of the extract (**Fig. 4**). There is a difference between the inhibitions of AE with Standard of about 14.54% at 0.5 mg/ml conc. while that of the ME is 1.37%, hence the activity of AE is less in comparison with ME (**Fig. 4**). % Inhibition of AE is higher than ME for *Thespesia* leaves extract. The % inhibition is of 96.9% at 0.5 mg/ml of ME. The inhibitory activity of AE is 22.67% less than the standard while that of ME is 2.87% less than the standard at 0.5mg/ ml concentration (**Fig. 5**). These results are in sync with the experimental results of [13] [24] [25] [26]. The AE shows less activity than that of standard and ME. The % Inhibition of ME is higher as compared to the AE for *Azadirachta* leaves and the difference in inhibition is about 11.7% at 0.5 mg/ml (**Fig. 6**). The comparative graph of *Azadirachta* AE, ME and Standard shows that at the maximum conc. (0.5 mg/ml) there is a difference of about 16.9% for the AE w.r.t. ME (**Fig. 6**). Such results were also obtained by various researchers like [8] [9] [20]. Standard while for that of ME the difference is about 4.2% w.r.t. Standard the ME had more inhibitory activity than AE (**Fig. 6**). The maximum IC_{50} values among the AE is of *Azadirachta* with 0.084mg/ml. The maximum IC_{50} values among the ME is of *Thespesia* with 0.06 mg/ml. On comparing both the extract (i.e., AE and ME) the highest IC_{50} values is of *Azadirachta* (**Fig. 7**). The IC_{50} values of AE are more than that of the ME. The IC_{50} values obtained for *Thespesia* is similar to that of results obtained [25]; while for *Azadirachta* results are almost similar to that [20].

CONCLUSION

The selected plants for the study have an extensive ethnobotanical importance hence, are probably having a wide range of phytoconstituents with pharmaceutical use in the coming future. Such phytochemicals in the leaves of these tree species that could trap the stable yet active compound DPPH,

then these plants will surely have the capabilities to scavenge those unwanted compounds produced everyday in living organisms. A wide range of research work will be required to explore and isolate the compounds that give such properties to our best friends –Plants.

Table 1
Phytochemical screening of *Azadirachta, Peltophorum and Eucalyptus*.

Sr. No	Phytochemical Screened	Test	*Azadirachta indica*		*Peltophorum pterocarpum*		*Eucalyptus globulus*	
			AE	ME	AE	ME	AE	ME
A	Alkaloids							
1.		Mayer's Test	P	P	P	P	P	P
2.		Dragendroff Test	P	P	P	P	P	P
3.		Wagner's Test	P	P	P	P	P	P
B	Flavonoid							
1.		Alkaline Reagent Test	P	A	P	P	P	P
2.		Shinoda Test	P	A	P	P	P	P
3.		Zinc Hydrochloride Reduction Test	P	P	P	P	P	P
4.		Pew Test	P	A	P	P	P	P
5.		Dilute Ammonia Test	P	P	P	P	P	P
C	Phenols							
1.		Ferric Chloride Test	A	P	P	P	P	P
2.		Lead Acetate Test	A	P	P	P	P	P
3.		Potassium Dichromate Test	A	P	P	P	P	P
4.		Alkaline Reagent Test	A	P	P	P	P	P
D	Tannins							
1.		Ferric chloride Test	A	P	P	P	P	P
2.		Lead acetate Test	P	P	P	P	P	P
3.		Potassium Dichromate Test	A	P	P	P	P	P
E	Saponins	Frothing Test	A	P	P	P	P	P
F								
1.	Steroids	Libermann - Buchard Test	A	A	A	A	A	A
2.		Acetic anhydride Test	A	A	A	A	A	A
3.		Libermann - sterol Test	A	A	A	A	A	A
G								
	Glycosides	Keller - Killiani Test	A	P	A	P	A	P

Table 2
Phytochemical screening of *Kigelia and Thespesia*.

S. No	Phytochemical Screened	Test	*Kigelia pinnata*		*Thespesia populnea*	
			AE	ME	AE	ME
A	Alkaloids					
1.		Mayer's Test	P	P	P	P
2.		Dragendroff Test	P	P	P	P
3.		Wagner's Test	P	P	P	P
B	Flavonoid					
1.		Alkaline Reagent Test	P	P	P	P
2.		Shinoda Test	P	P	P	P
3.		Zinc Hydrochloride Reduction Test	P	P	P	P
4.		Pew Test	P	P	P	P
5.		Dilute Ammonia Test	P	P	P	P
C	Phenols					
1.		Ferric Chloride Test	P	P	P	P
2.		Lead Acetate Test	P	P	P	P
3.		Potassium Dichromate Test	P	P	P	P
4.		Alkaline Reagent Test	P	P	P	P
D	Tannins					
1.		Ferric chloride Test	P	P	P	P
2.		Lead acetate Test	P	P	P	P
3.		Potassium Dichromate Test	P	P	P	P
E	Saponins	Frothing Test	P	P	P	P
F	Steroids					
1.		Libermann - Buchard Test	A	A	A	A
2.		Acetic anhydride Test	A	A	A	A
3.		Libermann - sterol Test	A	A	A	A
G	Glycosides	Keller - Killiani Test	A	P	P	P

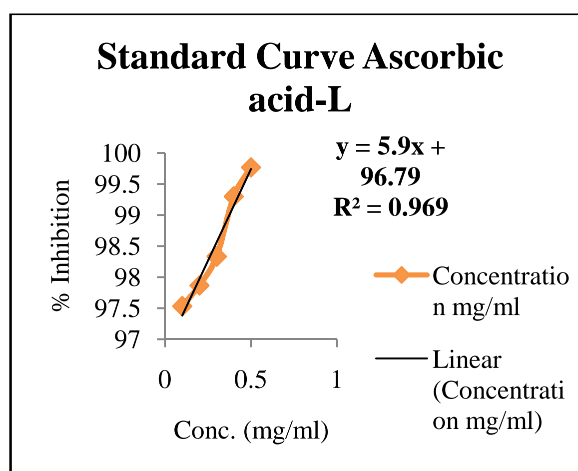

Fig. 1
Standard Curve Ascorbic Acid

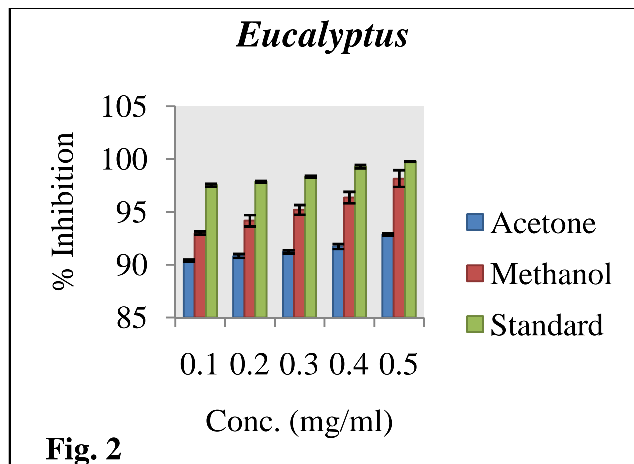

Fig. 2

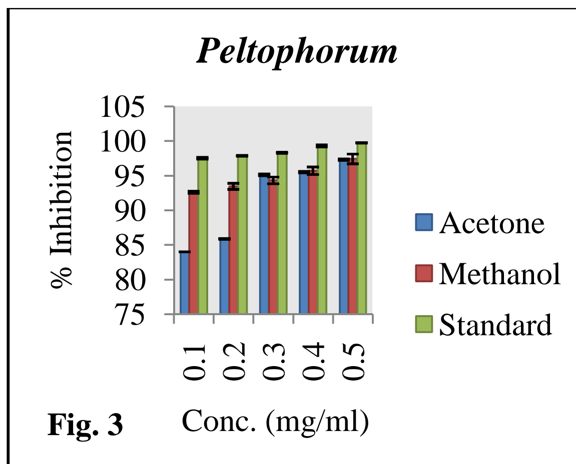

Fig. 3

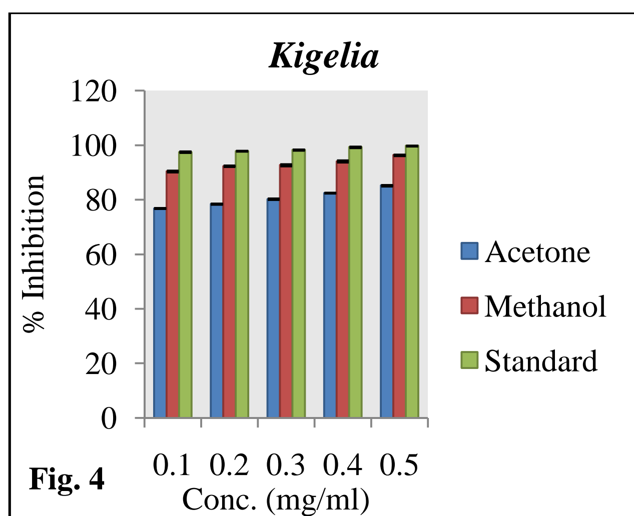

Fig. 4

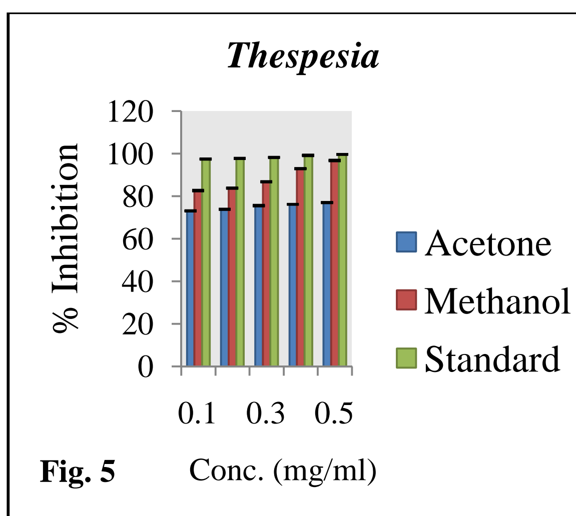

Fig. 5

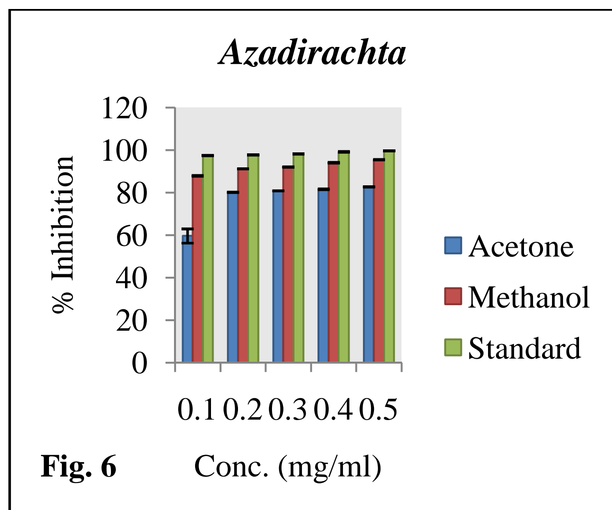

Fig. 6

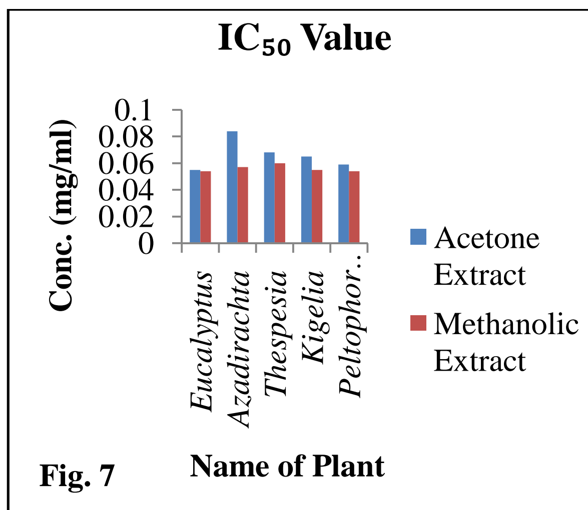

Fig. 7

Fig. 2-7

DPPH Assay of *Eucalyptus, Peltophorum, Kigelia, Thespesia* and *Azadirachta*; IC_{50} value of selected plants respectively (n=3)

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