#### TOTAL PHENOLIC CONTENTS AND ANTIOXDANT ACTIVITIES OF FICUS SYCOMORUS AND AZADIRACHTA INDICA

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#### Summary

In this study, the total phenolic content of the methanol, methanol-water mixtures and water extracts of the leaves of two plants growing in Egypt; Ficus sycomorus and Azadirachta indica were determined by using Folin-Ciocalteu reagent. Also, the antioxidant activity of these extracts was evaluated by using two methods including DPPH radical scavenging activity assay and total antioxidant capacity using phosphomolybdenum technique. The results showed that the antioxidant activities of the tested extracts were highly correlated with their total phenolic contents. Methanol (70 %) extract of each plant exhibited the highest antioxidant activity compared with other extracts in the two antioxidant methods. Therefore, the defatted methanol (70 %) extract was fractionated with certain organic solvents as CHCL<sub>3</sub>, EtOAc and n-BuOH, and then these fractions were submitted to antioxidant assessment .The butanolic fraction of each plant had the highest activity in the two antioxidant assays. Also, a linear positive correlation existed between the antioxidant activities of these fractions and their total phenolic and flavonoid contents. On the other hand, the effect of different temperatures (27, 50, 70 and 100 °C), different pH values (3, 5, 7, 9 and 11) and storage in the dark at 10 °C and 27 °C on the antioxidant activity of methanol extract of each plant was investigated. The results showed that the antioxidant activity of this extract of the two plants decrease with increasing the temperatures and exhibit the highest activity in neutral and acidic pH.Also storage of the methanol extract of each plant at 27 <sup>o</sup>C for one week did not show any significant change in the antioxidant activity whereas the activity decreased at  $10^{9}$ C. On the basis of these results, leaves of the two plants proved to be a good source of natural antioxidants due to their marked antioxidant activity.

Key Words: Phenolic, Antioxidant, DPPH, Plant extract.

#### Introduction

Reactive oxygen species (ROS) readily attach and induce oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA. This oxidative damage is a crucial etiological factor implicated in several chronic human diseases, namely cardiovascular diseases, rheumatism, diabetes mellitus and cancer (1). Based on growing interest in free radical biology and the lack of effective therapies for most chronic diseases, the usefulness of antioxidants in protection against these diseases is warranted. Antioxidants are chemical substances that reduce or prevent oxidation.

They have the ability to counteract the damaging effects of free radicals in tissues and thus are believed to protect against cancer ,arteriosclerosis ,heart diseases and several diseases (2,3). The most widely synthetic antioxidants ;butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been restricted recently because of serious concerns about their carcinogenic potential (4,5). Therefore there is great interest in finding new and safe antioxidants from natural sources. Numerous studies have been carried out on plant materials which resulted in the development of natural antioxidant formulations for food, cosmetic and other applications .However scientific information on antioxidant properties of various plants, particularly those that are less widely used in medicine is still scarce. Therefore the assessment of such properties remains an interesting and useful task, particularly for finding new sources for natural antioxidants, functional foods and nutraceuticals (6, 7).

Azadirachata indica commonly known as neem has attracted worldwide prominence in recent years owing to its wide range of biological and medicinal properties (8, 9, 10). There are many studies on the constituents of the neem tree including the limonoids, azadirachtin and nimbolide and several triterpens. Gallic acid, catechin, rutin and other phenolic and flavonoid compounds have been isolated from the neem has been isolated from different parts of A. indica (8, 9, 11, 12). On the other hand, different parts of *Ficus sycomorus* including fruits, leaves and fresh or dry tree bark are used for treatment of tumors and diseases associated or characterized by inflammation (13). Phytochemical investigations on a number of ficus species were undertaken and led to identification of several compounds where the major number of these compounds is phenolics and flavonoids (13, 14, 15).

The aim of this study was to determine the total phenolic content and the antioxidant properties of certain extracts of the leaves of two plants growing in Egypt. It is also of interest to evaluate the effect of different temperatures, different pH values and storage on the stability of the antioxidant activity of the most active extract of the two plant species which has not been previously investigated.

#### **Materials and Methods**

#### **Plant materials**

Leaves of *Azadirachta indica* A. Juss (Meliaceae) were collected from Faculty of Agriculture, Cairo University, Giza, Egypt in March 2006,whereas the leaves of Ficus sycomorus L.(Moraceae) were collected from El-Qualubia Governorate in March, 2007.The two plants were kindly identified by Prof. Dr. Wafaa Amer, Professor of Plant Taxonomy, Faculty of Science, Cairo University, Giza, Egypt. The fresh leaves of each plant was washed with clean water and completely dried in shade place at room temperature and then powdered by electric mill. The leaves powdered of each plant were stored at room temperature in dark places until subjected to the extraction process.

#### Chemicals

DPPH (1,1-diphenyl-2-picrylhydrazyl ) was purchased from Sigma-Aldrich Co., Also sodium phosphate ,ammonium molybdate, Folin - Ciocalteu's reagent, Ascorbic acid, and sodium carbonate were purchased from Merck chemical Co. All other chemicals and reagents were the highest analytical grade and purchased from common sources.

#### **Extraction and Fractionation**

Dried powdered leaves of each plant (100 g) were separately extracted with different solvents ;such as methanol ,methanol-water mixtures (90, 85,70 and 50 %) and distilled water room temperature (25 +2  $^{0}$ C) then each extract was filtered using Whattman filter paper no.1 and concentrated by using a rotatory evaporator. (Buchi, Switzerland).

The crude extracts were collected and stored at room temperature in the dark for the extraction process. The obtained crude methanol (70 %) extract (18 g) was defatted with petroleum ether and then fractionated by using different organic solvents; CHCl<sub>3</sub>, EtOAc and n-BuOH .Each fraction was filtered through filter paper Whatman no.1 and then concentrated by removing the solvent by using rotary evaporator. The yield of each fraction was measured and kept in dark for analysis.

#### Phytochemical screening

Identification of the major chemical constituents of the methanol (70%) extract was carried out using the standard procedures which previously described by Edeoga (16).

#### Determination of total phenolic content

The total phenolic content of each plant extract was determined using Folin–Ciocalteu reagent according to the method described by Kumar (17). Gallic acid was used as standard. In this method, the reaction mixture was composed of (100  $\mu$ l) of plant extract (100  $\mu$ l/ml) and 500  $\mu$ l of the Folin–Ciocalteu reagent and 1.5 ml of sodium carbonate (20 %). The mixture was shaken and made up to 10 ml using distilled water. The mixture was allowed to stand for 2 h. Then the absorbance was measured at 765nm using spectrophotometer (UV-VS spectrophotometer, Milton Roy 601).All determinations were carried out in triplicate .The total phenolic content was expressed as mg gallic acid equivalent (GAE) per g extract.

#### DPPH radical scavenging activity

The ability of each extract to scavenge DPPH radicals was measured according to the procedure described by Mensor (18). Briefly; 3 ml of each plant extract at a concentration of  $100 \mu g/$  ml was mixed with 1 ml of of 0.1 mM DPPH in methanol. The mixture was then shaken and left for 30 min at room temperature in the dark. The absorbance was measured at 517 nm using a spectrophotometer .Ascorbic acid was used as a reference standard. Control was prepared containing the same solvents and reagents without any extract and reference ascorbic acid. All experimental were carried out in triplicate. The activity of each extract was expressed as percentage DPPH radical scavenging activity.

The scavenging effect (antioxidant activity) of each extract was expressed as  $SC_{50}$  which defined as the concentration of each extract required for 50% scavenging of DPPH radicals compared with that of ascorbic acid which was used as the standard. The lower  $SC_{50}$  value corresponds to a higher scavenging activity (higher antioxidant activity) of plant extract.

#### Determination of total antioxidant capacity

The antioxidant activity of each plant extract was determined according to phosphomolybdenum method (19) using ascorbic acid as standard .In this method, 0.3 ml of each extract (100  $\mu$ g /ml) in methanol was combined in dried vials with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials containing the reaction mixture were capped and incubated in a thermal block at 95 °C for 90 min. After the samples had cooled at room temperature, the absorbance was measured at 695 nm against a blank. The blank consisted of all reagents and solvents without the sample and it was incubated under the same conditions. All experiments were carried out in triplicate. The antioxidant activity of the extracts was expressed as the number of equivalents of ascorbic acid (AAE).

#### Determination of total flavonoid content

The content of flavonoids of each extract was determined according to the reported procedures by Kumaran (20) using rutin as a standard. Briefly, 100  $\mu$ l of plant extract in methanol (100 mg/ml) was mixed with 100  $\mu$ l of aluminium trichloride in methanol (20 mg/ml) and then diluted with methanol to 500  $\mu$ l. The absorption at 415 nm was read after 40 min against the blank. The blank consisted of all reagents and solvents without any extract. All determinations were carried out in triplicate. The total flavonoid in plant extracts was determined as mg rutin equivalents (RE)/ g extract.

# Effect of different temperatures, different pH values and storage on the antioxident stability of the plant extract:

Studying the effect of temperature on the antioxidant activity of the methanol (70 %) extract of each plant under investigation according to the reported procedures by Arabshahi (21). Each extract was incubated at different temperatures (27, 50, 70 and 100  $^{0}$ C) for 30 min. After that the antioxidant activity was separately determined as previously mentioned. For pH stability, the extract was incubated at different pH values (3, 5, 7, 9 and 11) for 30 min, then antioxidant activity was separately evaluated. For storage stability; each plant extract was divided into two parts. The first part was stored in dark condition at 27  $^{0}$ C, whereas the other part was stored at 10  $^{0}$ C. Antioxidant activity of each part was determined after one week of exposure period.

#### Statistical analysis

*Experimental* results were expressed as mean  $\pm$  SD of three parallel measurements.using SPSS 13.0 Program. The SC<sub>50</sub> values were calculated using the SPSS 13.0 programme by probit-graphic interpolation for six concentration levels.Correlation analysis of the antioxidant activity versus the total phenolic were carried out using the correlation and regression by Microsoft Excel program 2003.

#### **Results and Discussion**

#### Extract yields and total phenolic content

The amount of the extract yields and the antioxidant activity of the plant extracts are strongly dependent on the polarity of the used solvents for the extraction process. It has been reported that mixtures of the methanol and water are the most widely employed solvents for extraction of the major antioxidant compounds from the plant materials. Also, the antioxidant activities of the extracts depend on the percent of the phenolic content in the tested extract (22, 23). In the present study, the methanol, methanol-water mixtures and water solvents are used for extraction of the chemical constituents of the two plant species; F.sycomorus and A.indica .The amount of the extractable substances of each extract was expressed as a percentage by weight of dried leaves of each plant. Also, the total phenolic content of each extract was estimated by using Folin-Ciocalteu reagent where their values were expressed as mg of gallic acid equivalent (GAE) per g extract. The results in tables 1 and 2 showed that the methanol, methanol-water mixtures and water have different abilities for extraction of different percents of extractable substances. The highest amount of the total extractable content (TEC) from the two plants was obtained by 70 % methanol (14.30 % for F.Sycomorus and 16.80 % for A.indica) followed by 85 % methanol (12.83 % for F.sycomorus and 16 % for A.indica). The other plant extracts gave different percentages of the extractable substances. Also, these results showed that; there are positive correlation between the total extractable content (TEC) and the total phenolic content (TPC) of each plant extract. As seen in tables 1 and 2 the highest amount of total phenolic content was found in methanol (70%) extract of each plant (229.32 mg gallic acid equivalent / g extract for F.sycomorus and 152.87 for A.indica ) followed by 85 % methanol, 90 % methanol, 50 % methanol and water extracts. The water extracts of the two plants gave the lowest amount of extractable substances and total phenolic contents .From these results ,it has been appeared that methanol (70 %) is preferred solvent for extraction of the chemical constituents of the two plants under investigation .The results are in full agreements with the previous studied which reported that the aqueous methanol is effective solvent for extraction of the phenolic compounds from selected tropical fruits and seeds of *Black cumin* (*Nigella sativa*) (23, 24). It has been reported that the higher contents of total phenolics in Ficus spp. might be contributed by presence of pelargonidin, leucopelargonin derivatives, flavonoids and high molecular tannins (5, 24, 25, 26). The major contributor of phenolics in A.indica might be quercetin ,rutin, gallic acid ,catechin and other phenolic and flavonoid compounds (27, 28).

#### DPPH radical scavenging activity

It is well known that the antioxidant activities of plant extracts which containing various phenolic compounds are due to their abilities to be donors of hydrogen atoms or electrons and to capture free radicals (29, 30). The DPPH radical-scavenging assay is a widely used method for evaluating the ability of plant extracts to scavenge free radicals generated from DPPH reagent. DPPH, a stable free radical with a purple colour, changes into a stable yellow compound on reacting with an antioxidant. In brief, the reduction capacity of DPPH was determined by the decrease in its absorbance at 517 nm, which is reduced by the antioxidant. The extent of the reaction depends on the hydrogen –donating ability of the antioxidant. The  $SC_{50}$  value of each extract is the concentration that required for 50 % scavenging of DPPH radicals under the conditions of each experimental. The lower SC<sub>50</sub> value corresponds to a larger scavenging activity (30, 31, 32). DPPH radical-scavenging activities (SC50 values) of methanol, methanol-water mixtures and water extracts of *F.sycomorus* and *A.indica* were determined. From the results in tables 1 and 2 it was appeared that the methanol (70 %) extract of each plant which containing the highest amount of total phenolic compounds has the strongest DPPH radical-scavenging activity (SC<sub>50</sub> =  $15.37 \mu g/ml$ for F.sycomorous and 32.0 µg/ml for A.indica) whereas the water extract of each plant gave the lowest DPPH radical scavenging activity (SC<sub>50</sub> = 66.58  $\mu$ g/ml for *F.Sycomorus* and 104.72  $\mu$ g/ml for A.indica). The other extracts showed variable activities against DPPH radical. Figures 1 and 2 showed that there is a positive correlation between the free radical scavenging activity of the tested extracts of the two plants and their total phenolic content. Correlation coefficients of F.Sycomorus and *A.indica* extracts against their total phenolic contents were  $R^2 = 0.77$  and 0.95 respectively. This correlation confirmed that the phenolic compounds are the major constituents contributing to the DPPH-scavenging activities of the extracts. These results are in full agreement with previous studies on other plant extracts which mentioned that the high level of total phenolic contents give low SC<sub>50</sub> values (24, 33, 34). The free radicals which are involved in the process of lipid peroxidation are considered to play a major role in numerous chronic pathologies such as cancer and cardiovascular diseases among others. Therefore, the ability of the plant extracts as free radical scavenger revealed that these extracts might used as new of natural antioxidants and prevent the reactive radical species from reacting biomolecules such as lipoproteins, polyunsaturated fatty acid (PUFA), DNA, amino acids, proteins and sugars in susceptible biological and food systems (34.,35).

#### Total antioxidant capacity

A wide range of assays can be used for assessment of the total antioxidant capacities of plant extracts. In the present work, the total antioxidant capacity of methanol, methanol-water mixtures and water extracts of *F.sycomorus* and *A.indica* were measured spectrophotometrically through phosphomolybdenum method which is based on the reduction of Mo (IV) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) compound with a maximum absorption at 695 nm. A high absorbance value of the sample indicates its strong antioxidant capacity. This method is a quantitative one, since the antioxidant capacity is expressed as the number of equivalent of ascorbic acid (19). The results in tables 1 and 2 exhibited that ,all the tested extracts of the two plant species showed considerable antioxidant capacities .Also methanol (70 %) extracts of the two plants exhibited high activity (317.08 mg ascorbic equivalent(AAE) / g extract of *F.sycomorus* and 231.39 mg (AAE)/g extract for *A.indica*). Figures 3 and 4 showed also strong relationships between the activity of the tested plant extracts and their total phenolic contents (Correlation coefficients;  $R^2 = 0.96$  and 0.94 for *F.sycomorous* and *A.indica* respectively). These results are in agreement with many previous studies which indicted a linear relationship between the antioxidant capacity of plant extracts and their total phenolic contents (22, 37, 38). Thus, the content of the phenolic compounds in each extract could be used as an important indicator of antioxidant capacity.

Owing to the high DPPH radical-scavenging activity and high total antioxidant capacity of the methanol (70 %) extract of *F.sycomorus* and *A.indica*. This extract was defatted and subjected to fractionation process by using organic solvents with different polarities such as chloroform, ethyl acetate and n-butanol .Total phenolic and flavonoid content, DPPH radical scavenging activity and total capacity of each fraction were evaluated. Results in tables 3 and 4 showed that the butanolic fraction of each plant has the highest total phenolic content (520 mg gallic acid equivalent; GAE / g extract for *F.sycomorus* and 440.48 mg / g for *A.indica*). Also, the high flavonoid content was recorded with the butanolic fraction of each plant (28.75 mg rutin equivalent; RE / g plant extract for *F.sycomorus* and 19.66 mg/g for *A.indica*).The total phenolic and flavonoids content of the other fractions decreased from ethyl acetate to chloroform for the two plant species.

Primary phytochemical screening of the most antioxidant active methanol (70 %) extract of each plant revealed that this extract contain certain bioactive agents which responsible for the antioxidant properties of this extract for each plant. These agents are flavonoids, tannins ,phenolic compounds and anthraquinones. , It has been reported that the phenolic and flavonoid compounds are associated with the antioxidative action in biological system acting as scavengers of singlet and free radicals (22, 39).

#### Temperature, pH and storage

It is known that, the activity of antioxidant extracts or compounds are strongly affected by many factors such as antioxidant concentration, temperature, pH and storage (21, 40). Therefore, the most antioxidant active methanol (70 %) extract was subjected to certain factors such as different temperatures (27, 50, 70, 100 °C), different pH values (3, 5, 7, 9, 11) and storage in the dark at 10 and 27 <sup>o</sup>C. The results in tables 5 and 6 showed that the antioxidant capacity of the tested extract is stable at 27 °C whereas it decrease with increasing the temperatures from 50 °C up to 100 °C. This decreasing in the activity at high temperatures might be related to either loss of naturally occurring antioxidants present in the tested extract or formation of novel compounds having prooxidant activity (21, 40). Azizah,(41) found that the antioxidant activities of the extracts from six types of Cocoa by -products are stable at temperatures below 50  $^{\circ}$ C and this activity decreased by increasing the temperatures at 70-90 °C. Similarly, Juntachote and Berghofer (42) reported that the ethanol extract of Holy basil and Galangal showed good heat stability up to 80 °C for 60 min. Furthermore, the antioxidant activities of Ginger extracts showed good thermal stability (43). The influence of pH on the antioxidant activity of methanol (70 %) extract of each plant was studied and the results in tables 3 and 4 showed that the activity of the extract is table at neutral and acidic pH whereas this activity decreased in alkaline pH .This results demonstrated that the antioxidant activity of the tested extract is strong dependence pH of the system (4). It has been reported that the extracts from Peanut hull and Aspergillus candidus exhibited strong antioxidant activity at neutral and acidic pH range but rendered no activity at alkaline pH range (44, 45). This is contrary to the study done by Azizah (41). Who reported that the antioxidant activities of different extracts from six types of cocoa by-products exhibit highest activities at neutral and alkaline pH. The difference in the antioxidant activities of plant extracts at various pH values is possibly due to the different samples and different compounds being extracted in each plant.

The effect of storage on the stability of the antioxidant activity of the methanol (70 %) extract at 10  $^{0}$ C and 27  $^{0}$ C was also studied .It has been found that the activity did not show any change at 27  $^{0}$ C but exhibited least activity at 10  $^{0}$ C.

MeOH 90

% MeOH 85

% MeOH 70

% MeOH 50

%

 $H_2O$ 

10.44

12.83

14.30

7.36

4.06

F.sycomorus. TPC DPPH SC<sub>50</sub> TEC Total Extract % (mg GAE / g antioxidant capacity of  $[\mu g/ml]$ (mg AAE/g ext.)ext.) 11.90  $151.49 \pm 3.03$ MeOH  $124.00\pm4.96$  $20.93 \pm 0.21$ 

 $18.87\pm0.09$ 

 $17.30\pm0.55$ 

 $15.37\pm0.07$ 

 $30.39 \pm 0.91$ 

 $66.58 \pm 0.75$ 

 $181.79\pm3.03$ 

 $224.23 \pm 13.88$ 

 $317.08 \pm 13.53$ 

 $84.81\pm3.03$ 

 $30.29 \pm 3.03$ 

 $150.99\pm2.87$ 

 $188.59 \pm 2.86$ 

 $229.32 \pm 4.97$ 

 $98.36 \pm 4.73$ 

 $26.31 \pm 3.76$ 

Table-1. Total extractable content (TEC), Total phenolic content (TPC), Free radical scavenging potential (DPPH) and total antioxidant capacity of the different extracts of *F.sycomorus*.

Results are means  $\pm$  SD (n = 3),TEC (total extractable content); TPC (total phenolic content); GAE (gallic acid equivalent), AAE (ascorbic acid equivalent).

Table-2. Total extractable content (TEC), Total phenolic content (TPC), Free radical scavenging potential (DPPH) and total antioxidant capacity of the different extracts of *A.indica* 

Extract	TEC	TPC	DPPH SC <sub>50</sub>	Total antioxidant
of	%	(mg GAE / g	[µg/ml]	capacity (mg AAE /g
		ext.)		ext.)
MeOH	12.84	$92.10 \pm 3.76$	$71.52\pm0.41$	$113.11 \pm 3.49$
MeOH 90 %	11.46	$112.78 \pm 3.76$	$58.54 \pm 0.11$	$146.43 \pm 1.74$
MeOH 85 %	16.00	$130.94\pm2.87$	$53.52 \pm 1.02$	$157.54 \pm 6.06$
MeOH 70 %	16.80	$152.87\pm7.59$	$32.00\pm0.29$	$231.39\pm4.75$
MeOH 50 %	8.40	$57.64 \pm 2.87$	76.58 ±	$63.61 \pm 3.03$
			1.66	
H <sub>2</sub> O	6.12	9.39 ± 1.88	$104.72 \pm$	$12.10 \pm 3.06$
			0.83	

Table-3.Total extractable content (TEC), Total phenolic content (TPC), Free radical scavenging potential (DPPH), Total Flavonoids and total antioxidant capacity of the defatted methanol (70 %) extract of *F.sycomorus* and its fractions.

Extract of	TEC %	TPC (mg GAE / g ext.)	TF (mg RE / g ext.)	DPPH SC <sub>50</sub> [µg/ml]	Total antioxidant capacity (mg AAE /g ext.)
Defatted MeOH	4.54	254.37 ± 1.08	$14.19 \pm 1.01$	$13.55 \pm 0.08$	359.27 ± 2.99
CHCl <sub>3</sub>	2.52	180.79 ± 1.88	$4.66\pm0.66$	132.41 ± 1.17	$121.21 \pm 6.06$
EtOAc	0.83	280.19 ± 3.76	$18.76 \pm 1.08$	$13.48 \pm 0.12$	$370.70 \pm 7.06$
n-BuOH	1.20	$520.00 \pm 2.87$	$28.75 \pm 0.51$	8.47 ± 0.13	439.23± 8.85

RE = rutin equivalen	RE=	rutin	eq	uiv	al	en
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Table-4. Total extractable content (TEC), Total phenolic content (TPC), Free radical scavenging potential (DPPH), Total Flavonoids and total antioxidant capacity of the defatted methanol (70%) extract of *A.indica* and its fractions.

Extract of	TEC %	TPC (mg GAE / g ext.)	TF (mg RE / g ext.)	DPPH SC50 [µg/ml]	Total antioxidant capacity (mg AAE /g ext.)
Defatted MeOH	11.38	$169.79 \pm 2.87$	$7.54 \pm 1.06$	30.91± 0.54	264.46 ± 1.68
CHCl <sub>3</sub>	3.16	80.13 ± 1.08	$2.44\pm0.38$	$166.55 \pm 1.24$	58.58 ± 1.74
EtOAc	0.07	$200.04 \pm 2.87$	$10.98 \pm 0.68$	28.16 ±0.36	$279.79 \pm 7.62$
n-BuOH	4.43	440.48 ± 2.87	$19.66 \pm 0.3$	$21.08 \pm 0.14$	393.93±3.03

Table-5. Effect of dfferent temperatures on the Total antiox	kidant Capacity of the methanol
(70%) extract of F.Sycomorus and A.indica.	

Sample	Total antioxidant capacity (mg AAE /g ext.)					
Temp. °C	27 °C	50 °C	70 °C	100 °C		
F.Sycomorus	317.08 ± 13.53	143.71 ± 7.92	30.93 ± 1.70	10.97 ± 1.72		
A.indica	231.39 ± 4.75	97.80 ± 9.14	17.96 ± 5.18	3.98 ± 1.69		

Table- 6. Effect of different pH values on the Total antioxidant Capacity of the methanol (70 %) extract of *F.Sycomorus* and *A.indica* 

Sample	Total antioxidant capacity (mg AAE /g ext.)							
pH values	3	5	7	9	11			
F.Sycomorus	364 ± 6.02	334.33 ± 5.21	187.74±7.58	93.36 ± 6.32	35.13 ± 3.47			
A.indica	289.15 ± 6.30	247.08 ± 5.38	142.56 ± 6.11	42.16 ± 5.01	15.05 ± 3.01			



Fig -1. Correlation between DPPH radical scavenging activities of the different extracts of F. *sycomorous* and their total phenolic contents .



Fig -2. Correlation between DPPH radical scavenging activities of the different extracts of A. *indica* and their total phenolic contents.



Fig -3. Correlation between the total antioxidant capacity of the different extracts of *F. sycomorous* and their total phenolic contents.



Fig -4. Correlation between the total antioxidant capacities of the different extracts of extracts of *A. indica* and their phenolic contents.

#### Conclusion

Various solvent extracts from *F.sycomorous and A.indica* leaves showed varying degrees of antioxidant activities .This activity was correlated with the amount of total phenolics present in the respective extracts in each case. The methanol (70%) extract which contain the highest amount of phenolic compounds exhibited the strongest antioxidant activity in all assays. Therefore, methanol (70%) extract proved to be the most efficient solvent for extraction antioxidant compounds from the two plants .The activity of this extract varied with different PH values, different temperatures and storage .The results of the present study certainly help to ascertain the potency of the crude extracts of the leaves of the two plants as potential source of natural antioxidants. However, further studies are needed to identify the individual components which forming the antioxidant activity of the two plants.

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