

Comparative Total Phenolic Content and Antioxidant Activity of *Sida cardifolia*, *Abutilon indicum* and *Mesua ferrea*

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ABSTRACT

Background: Phenolic constituents of plant are the prime source of antioxidants. Natural antioxidant biomolecules become an emerging research area of this era because of their antioxidant potential. Accumulation of free radicals results in occurrence of many degenerative diseases and produced harmful effect on body. Research evidence proved that plant phenolic constituents have an antioxidant potential and has been used for the treatment of degenerative diseases. Therefore, an attempt was taken to investigate the antioxidant activity and total phenolic content of *Sida cardifolia*, *Abutilon indicum* and *Mesua ferrea*. **Materials and Methods:** Alcoholic, hydroalcoholic and water extracts of *S. cardifolia*, *A. indicum* and *M. ferrea* were prepared and total phenolic content was determined by Folin Ciocalteu method, and antioxidant activity was determined by DPPH free radical scavenging method. **Results:** Poly phenolic Result revealed that hydroalcoholic extracts of *S. cardifolia*, *A. indicum* and *M. ferrea* showed maximum phenolic content than the alcoholic and water extracts. *In vitro* Antioxidant study revealed that hydroalcoholic extract of *S. cardifolia* and *M. ferrea* showed optimum antioxidant activity while aqueous extract of *A. indicum* showed maximum antioxidant activity. **Conclusion:** Aqueous extract of *A. indicum* showed maximum antioxidant activity than hydroalcoholic extract of *S. cardifolia* and *M. ferrea*.

Keywords: Polyphenolic constituents, Biomolecules, Free radicals, Oxidative stress, Degenerative diseases.

INTRODUCTION

Free radicals are endogenously produced in the body due to formation of ROS (reactive oxygen species), OH⁻ (reactive hydroxyl radical), O₂ (superoxide anion radical), H₂O₂ (hydrogen peroxide), O₂(singlet oxygen), NO (nitric oxide radical), etc., and exogenously produced by smoking, alcoholism, pollution, organic solvents, and UV rays. They prominently attack on nucleic acid, proteins, enzymatic system, and other small molecules damaging DNA, causing loss of structure and functions of cells and system.¹⁻² Oxidative stress produced by free radicals causes degenerative disorders like cancer, diabetes, Alzheimer's disease, cardiac disorders, Parkinson's disease, atherosclerosis, aging, mutagenesis, gastric ulcer, etc. Indigenous antioxidant system combats free radicals and protect the body from the harmful effect of oxidative stress but the accumulation of free radicals due to overproduction destroy the antioxidant system causes imbalance and produces diseases. Antioxidant play crucial role in combating free radicals and protect the body from the harmful effect of oxidative stress. Research studies proved that the dietary intake of antioxidants play crucial role in the management and prevention of many diseases associated with oxidative stress of free radicals.³⁻⁴ Natural antioxidant present

in whole grains, fruits, flowers, seeds, leaves, plants have been reported to control the production of free radicals and protect the body from the damaging effect of free radicals. Natural phytoconstituents like Vitamin C, Vitamin E, carotenoids, ellagic acid, phenolic acids, polyphenols, flavonoids are the good source of natural antioxidant protect and prepare the body to fight against the oxidative damage (Figure 1).⁵⁻⁶ Polyphenolic constituents of plants is the good source of antioxidant which acts as free radical scavenging potential to protect the body from the occurrence of harmful diseases associated with free radicals.⁷ Sources of antioxidant is summarized in Figure 2. Research evidence proved that plant phenolic constituents have an antioxidant potential and has been used for the treatment of degenerative diseases. Therefore, an attempt was taken to investigate the antioxidant activity and total phenolic content of *Sida cardifolia*, *Abutilon indicum* and *Mesua ferrea*.

MATERIALS AND METHODS

Plant material of *S. cardifolia*, *A. indicum* and *M. ferrea* were purchase from the market, dried and powdered. The extracts of both drugs were prepared by the cold maceration method for 24 – 48 hr with

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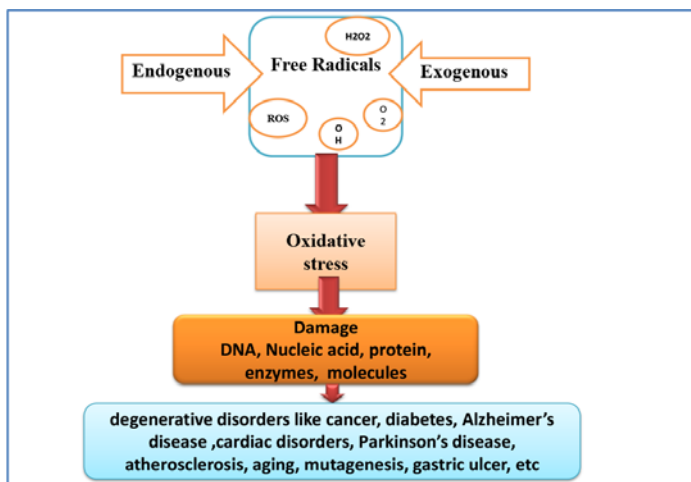


Figure 1: Schematic diagram of oxidative damage.

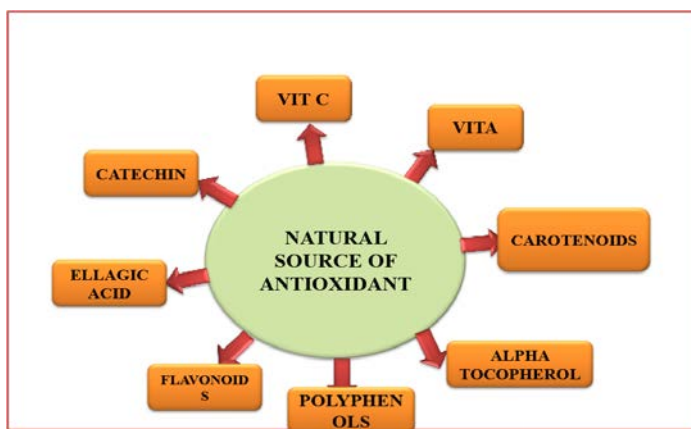


Figure 2: Sources of natural antioxidants.

occasional stirring at room temperature. 50 g of each dried powdered drug was transferred to the conical flask. 200 ml of solvent was added and kept for maceration for 24 hrs with occasional shaking, after 24 hrs, the extract was filtered, and mark was transferred to the conical flask and again 200 ml of solvent was added and kept for maceration for 24 hr. Again, the extract was filtered, filtrates were combined and concentrated at temperature below 500 C. Dried extracts were used for the total phenolic, and antioxidant study.

Three types of extracts were prepared with following solvent composition

Sl. No	Type of extract	Code	Solvents	Composition
1	Alcoholic	A	95% ethanol	100%
2	Hydro alcoholic	HA	70% ethanol	70 ml ethanol + 30 ml water
3	Water	W	100%	100% water

Determination of Total phenolic content by Folin-Ciocalteu Method

Total phenolic content of alcoholic, hydroalcoholic and water extracts of *S. cardifolia*, *A. indicum* and *M. ferrea* were determined with Folin-Ciocalteu method.⁸ Standard solution of Gallic acid (10-100 µg/ml) in water was prepared. Stock solution of extracts 1mg /ml in water were prepared. 1 ml of each sample were mixed with 0.25 ml of Folin-Ciocalteu reagent and 1.25 ml of 20% sodium carbonate solution. Allow

the mixture to react for 40 min at room temperature in dark place.⁹ After the reaction period, absorbance was measured at 760 nm in comparison with standards and the total phenolic content was calculated as gallic acid, equivalents and expressed in % as gallic acid, shown in Table 1.

Determination of Antioxidant activity of the plant extracts by DPPH method

Sample extract at various concentrations were taken and the volume were adjusted to 100 µL with methanol. A stock solution of DPPH (33 mg in 1 l) was prepared in methanol; 5 mL of methanolic solution of DPPH (0.1 mM) was added to 1 ml of extract solution at different concentration and shaken vigorously. The tubes were allowed to stand for 20 min at 27°C.¹⁰⁻¹¹ The absorbance of the sample were measured at 517 nm. Antioxidant activity result shown in Table 2.

RESULTS AND DISCUSSION

Phenolic content of alcoholic, hydroalcoholic and water extracts of *S. cardifolia*, *A. indicum*, and *M. ferrea* were determined by Folin-Ciocalteu method. Antioxidant activity of alcoholic, hydroalcoholic and water extracts of *S. cardifolia*, *A. indicum*, and *M. ferrea* were determined by DPPH method. Folin-Ciocalteu method it also called as gallic acid equivalence method, it is an antioxidant assay based on electron transfer, which measures the reductive capacity of an antioxidant. This method has been widely applied in determination of the total phenol and polyphenol content of herbal extracts or phytochemicals. It is precise, accurate, reproducible, and easy to perform. DPPH (α, α-diphenyl-β-picrylhydrazyl free radical scavenging method use for evaluation of the antioxidant potential of a plant compound, which is the simplest, precise, and accurate method, in which extract is mixed with DPPH solution and absorbance is recorded after a defined period.

Total Phenolic Content

Table 1: Total Phenolic content of Alcoholic (A), Hydro alcoholic (HA) and Water(W) extracts of *S. cardifolia* (S), *A. indicum* (A) and *M. ferrea* (M).

Name of Sample	Sample code	Absorbance	% Phenolic content
<i>S. cardifolia</i>	SA	0.17	2.62
	SHA	0.439	8.69
	SW	0.339	6.43
<i>Indicum</i>	AA	0.262	4.70
	AHA	0.319	5.98
	AW	0.311	5.80
<i>M. ferrea</i>	MA	0.789	17.58
	MHA	1.226	25.43
	W	0.689	15.32

CONCLUSION

Total phenolic content of alcoholic, hydroalcoholic and water extract of *S. cardifolia*, *A. indicum*, and *M. ferrea* were found (2.62,8.69,6.43); (4.70,5.98,5.80); and (17.58,25.43,15.32) respectively. Antioxidant activity of alcoholic, hydroalcoholic and water extract of *S. cardifolia*, *A. indicum*, and *M. ferrea* were found (40%,40.59%,36.42%); (35.88%,39.55%,40.14%); and (59.85, 91.04, 40.14,) respectively. From the result it is revealed that Hydroalcoholic extract contain highest total phenolic content, than the alcoholic and water extract. *In vitro* Antioxidant study revealed that hydroalcoholic extract of *S. cardifolia* and *M. ferrea* showed optimum antioxidant activity while aqueous extract

Table 2: Antioxidant activity of alcoholic, hydroalcoholic, and Water extracts of *S. cardifolia*, *A. indicum* and *M. ferrea*.

Sl. No	Name of Sample	Sample Code	Conc. (µg/ml)	Abs	% Inhibition
1	<i>S. cardifolia</i>	Alcoholic	1	0.442	34.02%
			2	0.434	35.22%
			3	0.416	37.91%
			4	0.407	39.25%
			5	0.402	40%
		Hydroalcoholic	1	0.449	32.98%
			2	0.444	33.73%
			3	0.429	35.97%
			4	0.401	40.14%
			5	0.398	40.59%
		Water	1	0.497	25.82%
			2	0.474	29.25%
			3	0.450	32.83%
			4	0.431	35.67%
			5	0.426	36.41%
2	<i>Indicum</i>	Alcoholic	1	0.515	23.14%
			2	0.502	25.07%
			3	0.479	28.50%
			4	0.466	30.44%
			5	0.430	35.88%
		Hydroalcoholic	1	0.467	30.29%
			2	0.456	31.94%
			3	0.446	33.43%
			4	0.426	36.41%
			5	0.405	39.55%
		Water	1	0.492	26.56%
			2	0.455	32.08%
			3	0.440	34.34%
			4	0.425	36.56%
			5	0.401	40.14%
3	<i>M. ferrea</i>	Alcoholic	1	0.383	42.83%
			2	0.350	47.76%
			3	0.324	51.64%
			4	0.294	56.11%
			5	0.269	59.85%
		Hydroalcoholic	1	0.404	39.70%
			2	0.209	68.80%
			3	0.116	82.68%
			4	0.069	89.70%
			5	0.060	91.04%
		Water	1	0.457	31.79%
			2	0.455	32.08%
			3	0.427	36.26%
			4	0.407	39.25%
			5	0.401	40.14%

of *A. indicum* show maximum antioxidant activity. *In vitro* antioxidant study revealed that the *S. cardifolia*, *A. indicum*, and *M. ferrea* showed remarkable antioxidant effect due to their phenolic contents. Free radicals produced in biological systems by the oxidative stress, causes DNA damage and are responsible for degenerative disorders, like aging, mutagenesis, carcinogenesis, and cardiovascular disorder. Polyphenolic plant constituents have the antioxidant potential, which combat the free radicals. This study revealed that the *S. cardifolia*, *A. indicum*, and *M. ferrea* would be the good source of antioxidant due to its polyphenolic content which can be used in combination for the treatment of many diseases associated with free radicals. In combination they would produce synergistic effect, further study will be carried out in combination.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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