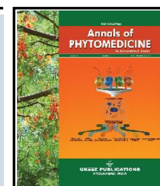
Annals of Phytomedicine 11(2): 507-515, 2022

DOI: http://dx.doi.org/10.54085/ap.2022.11.2.62

Annals of Phytomedicine: An International Journal

http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Phytochemical analysis and *in vitro* antioxidant studies of the leaf extracts of some Indian medicinal plants from western Maharashtra

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Article Info

Article history
Received 5 August 2022
Revised 22 September 2022
Accepted 23 September 2022
Published Online 30 December-2022

Keywords
Aegle marmelos L. Correa.
Cynodon dactylon L. Pers.
Annona squamosa Linn.
Hibiscus rosa-sinensis Linn.
Preliminary phytochemical analysis
In vitro antioxidant activity
Medicinal plants

Abstract

Objective of the present study was to perform phytochemical and *in vitro* antioxidant analysis of the leaf sample extracts of *Aegle marmelos* L Correa., *Cynodon dactylon* L. Pers., *Annona squamosa* Linn. and *Hibiscus rosa-sinensis* Linn. to understand their potential medicinal benefits.

Preliminary phytochemical screening of the plant leaf extracts was performed. *In vitro* antioxidation parameters like reducing power, nitric oxide radical, superoxide anion radical and hydroxyl radical scavenging assays were performed to evaluate the antioxidant potential of the leaf extracts in methanol. Determination of the total concentration of phenolic and flavonoid compounds was also done. The findings showed the presence of alkaloids, tannins, saponins, flavonoids, glycosides, phenolic compounds, terpenoids and steroids. The results of the study revealed that the leaf extracts of *A. marmelos*, *C. dactylon*, *A. squamosa* and *H. rosa-sinensis* also contain phytochemicals and antioxidants in good amount. The leaf extracts of *A. marmelos*, *C. dactylon*, *A. squamosa* and *H. rosa-sinensis* can prove to be easily accessible source of natural antioxidant, thus making it a good choice as a promising food supplement or in pharmaceutical industry.

1. Introduction

Secondary metabolites of medicinal plants contribute to their clinically beneficial effects and are also important indicators for evaluating the quality of medicinal materials (Singh *et al*., 2022; Yanqun *et al.,* 2020). Traditional formulation of medicinal plants is used since ancient times. Majority of the world's inhabitants are dependent on such medicinal plants for the treatment of various diseases and for primary healthcare system. Many of the currently used drugs have been developed using the information of indigenous knowledge of these medicinal plants (Pant, 2014).

Antioxidants play a major role in neutralizing free radicals and reactive oxygen species (ROS). Different plant phenols like flavonoids, curcumin, stilbenes, catechins, eugenols, *etc*., are effective antioxidants and possess the capacity to protect cells during oxidative stress. Many research studies are being conducted to study the potential use of phenolic compounds for treatment of cardiovascular, neurodegenerative, oncological and inflammatory diseases (Mehrotra, 2021; Zenkov *et al.,* 2016).

Phenolic components in the plants, such as flavonoids, phenolic acids and phenolic diterpenes majorly contribute to its antioxidative properties. Due to their redox properties, phenolic compounds absorb and neutralize the free radicals and also decompose the peroxides, thereby inhibiting the lipid peroxidation (Panovskai *et al.,* 2005). All parts of plants such as leaves, fruits, seeds, roots and bark have these components (Mathew *et al*., 2019; Mathew *et al.,* 2000).

Secondary metabolites like polyphenols of plants are generally involved in the plants defense against radiations, especially ultraviolet and also are involved in protecting plants from the pathogens. Now-a-days, studies are focusing on obtaining the potential health benefits of dietary plant polyphenols as antioxidants. As per the different epidemiological studies, it has been proposed that diet rich in plant polyphenols has protective effects against development of various diseases like, diabetes, respiratory disorders, osteoporosis and different neurodegenerative diseases (Kaushal *et al*., 2022; Abubucker *et al*., 2022; Pandey *et al.,* 2009).

Several natural food sources and plants have been evaluated for their antioxidant potential, especially phenolics and flavonoids. Also many other plant species have been investigated for their antioxidant capacity, but still there is demand to find more information regarding the antioxidant potential of plant species as they are safe and also bioactive (Patel *et al.,* 2010). Much effort with more research studies are, therefore required to a search for the potential antioxidants from both, synthetic and from natural sources, mostly plants (Pinchuk *et al.,* 2012). Hence, in the present study, we aim to conduct phytochemical analysis and *in vitro* antioxidant potential of the leaf powder of *A. marmelos*, *C. dactylon*, *A. squamosa* and *H. rosa-sinensis*.

2. Materials and Methods

Leaves of *Aegle marmelos* L. Correa (Acc. No.-08649, 08652), *Cynodon dactylon* L. Pers. (Acc. No.- 83143), *Annona squamosa* Linn. (Acc. No.-102268, 00469) were collected from Mumbai and

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Talegaon Dabhade (district - Maval, Pune). Authentication of all the plant samples under the study was done by Dr. Rajendra Shinde, Blatter Herbarium, St. Xavier's College, Mumbai.

2.1 Phytochemical screening

For screening of various phytochemicals, around 5 g each of the *A. marmelos* leaf powder, *C.dactylon* leaf powder, *A.squamosa* leaf powder and *H. rosa-sinensis* powder were extracted. The rotory shaker was used for eight hours and extraction was done with 100 ml of methanol. Similarly, the extraction was also done using water. The extract was filtered and then concentrated by evaporating it. The presence of alkaloids, tannins, saponins, flavonoids, glycosides, phenolic compounds, terpenoids, steroids and reducing steroids was detected using known and standardized qualitative assays (Pawaskar and Sasangan, 2017).

2.2 *In vitro* antioxidant study

5 g each of the *A. marmelos*, *C. dactylon*, *A. squamosa* and *H. rosa-sinensis* leaves were weighed. Continuous extraction on rotary shaker using 95% methanol (volume-250 ml) for about 18-20 h was done. Concentration of the extracts was done and then drying was done in controlled temperature (40-50°C)and under reduced pressure. The residues were weighed and stored in in sealed vials in a freezer until tested. The extracts were reconstituted in water to prepare the stock solution of required concentration, which were then diluted and used for the *in vitro* antioxidation assays.

The assays for total antioxidant capacity, total phenol content, total flavonoid content (flavones and flavonols), reducing power, scavenging ability for hydroxyl radicals, nitric oxide radical scavenging, superoxide anion scavenging activity were performed using known and standardized methods (Pawaskar and Sasangan, 2017).

3. Results

3.1 Phytochemical screening

The results ofscreening of various phytochemicals from the leaf extract of *A. marmelos*, *C. dactylon*, *A. squamosa* and *H. rosa-sinensis* have been shown in Table 1.

Table 1: Results of screening of various phytochemicals from the leaf extract of *A. marmelos, C. dactylon, A. squamosa and H. rosa-sinensis* in different solvents

S. No.	Phytochemicals	PE	CL	EA	AC	ET	ME	WT
	***Aegle marmelos* L. Correa**							
1	Terpenoids	-	+	-	+	+	+	-
2	Steroids and phytosterols	+	+	-	+	+	+	-
3	Tannins	+	+	-	+	+	-	+
4	Alkaloids	+	+	+	+	+	+	+
5	Glycosides	-	+	+	+	+	+	+
6	Saponins	-	-	-	+	+	-	+
7	Phenols	-	-	-	-	+	-	+
8	Flavonoids	-	-	-	-	+	+	-
9	Reducing sugars	-	-	-	-	-	+	+
	***Cynodon dactylon* L. Pers.**							
1	Terpenoids	-	-	-	+	+	+	-
2	Steroids and phytosterols	+	+	-	+	+	+	-
3	Tannins	+	+	-	+	+	-	+
4	Alkaloids	+	+	+	+	+	+	+
5	Glycosides	-	+	+	+	+	+	+
6	Saponins	-	-	-	-	+	-	+
7	Phenols	-	-	-	-	+	+	+
8	Flavonoids	-	-	-	-	+	+	-
9	Reducing sugars	-	-	-	-	+	+	+
	***Annona squamosa* Linn.**							
1	Terpenoids	-	+	-	+	+	+	-
2	Steroids and phytosterols	+	+	-	+	+	+	-
3	Tannins	-	+	-	+	+	-	+
4	Alkaloids	+	+	+	+	+	+	+

5	Glycosides	-	+	+	+	+	+	+
6	Saponins	-	-	-	-	-	-	+
7	Phenols	-	-	-	-	+	-	+
8	Flavonoids	-	+	-	-	+	-	+
9	Reducing sugars	-	-	-	-	+	+	+
	***Hibiscus rosa-sinensis* Linn.**							
1	Terpenoids	-	-	-	+	+	+	-
2	Steroids and phytosterols	+	+	-	+	-	-	-
3	Tannins	-	+	-	+	+	+	+
4	Alkaloids	-	+	+	+	+	+	+
5	Glycosides	-	+	+	+	+	+	-
6	Saponins	-	+	-	-	-	-	+
7	Phenols	-	+	-	-	+	+	+
8	Flavonoids	-	+	-	-	+	+	+
9	Reducing sugars	-	-	-	-	+	+	+

(PE-Petroleum ether; CL-Chloroform; EA-Ethyl acetate; AC-Acetone; ET-ethanol; ME-Methanol; WT-Water).

In the present study, presence of tannins, terpenoids, steroids and phytosterols, alkaloids, glycosides, saponins, reducing sugars, phenols and flavonoids has been found in all the plant extracts. Effective extraction of several phytochemicals was seen more in polar solvents like ethyl alcohol, methyl alcohol and water than the nonpolar solvents. Especially, leaf sample extracts of all the above mentioned plants in ethyl alcohol showed presence of most of the tested phytochemicals. Hence, it can be stated that alcohols can be the best solvents for the extraction of active principle than others.

So also in the present study, steroids, tannins, alkaloids, glycosides, saponins, phenols and flavonoids were detected in the chloroform extracts of *H. rosa-sinensis* leaves. Except steroids and saponins, the methonalic extracts of *H. rosa-sinensis* showed the presence of tannins, alkaloids, glycosides, phenols, flavonoids and reducing sugars.

Overall, considerably high amounts of most of the phytochemicals were detected in the leaf powder extracts of *A. marmelos*, *C. dactylon*, *A. squamosa* and *H. rosa-sinensis*. Considering wide range of medicinal and therapeutic uses and the future demand of these phytochemicals, it can be thus concluded that our study has highlighted the applicability and importance of the identified plants for medicinal use. These plant species under our study could also be seen as probable sources of useful drugs in futuredue to their good content of phytochemicals.

3.2 *In vitro* antioxidant study

The results of various antioxidation studies are discussed below:

3.2.1 Total antioxidant activity (TAA)

Table 2: Total antioxidant activity (TAA) expressed as mg of gallic acid equivalents (GAE)/g of the plant material (leaf powders of *A. marmelos, C. dactylon*, *A. squamosa* and *H. rosa-sinensis*)

S. No.	Plant sample	Total antioxidant activity (TAA) expressed as mg of gallic acid equivalents (GAE)/g of the plant material
1	AML	2.709 ± 0.08
2	CDL	3.482 ± 0.12
3	ASL	3.833 ± 0.10

*All the values are expressed as mean ± SD after three determi-nations

3.2.2 Total phenolic content (TPC)

Table 3: Total phenolic content (TPC) expressed as mg of gallic acid equivalents (GAE)/g of the plant material (leaf powders of *A. marmelos, C. dactylon*, *A. squamosa* and *H. rosa-sinensis*)

S. No.	Plant sample	Total phenolic content (TPC) expressed as mg of gallic acid equivalents (GAE)/g of the plant material
1	AML	4.402 ± 0.11
2	CDL	5.342 ± 0.09
3	ASL	5.693 ± 0.12
4	HRS	26.32 ± 0.24

*All the values are expressed as mean ± SD after three determinations.

3.2.3 Total flavonoid content (TFC)

Table 4: Total flavonoid content (flavones and flavonols)-(TFC)-expressed as mg of the plant material (leaf powders of *A. marmelos, C. dactylon*, *A. squamosa* and *H. rosa-sinensis*)

S. No.	Plant sample	Total flavonoid content (flavones and flavonols)-(TFC)-expressed as mg of quercetin equivalents (QE)/g of the plant material
1	AML	8.360 ± 0.10
2	CDL	8.661 ± 0.11
3	ASL	5.693 ± 0.06
4	HRS	17.58 ± 0.19

*All the values are expressed as mean ± SD after three determinations.

3.2.4 Reducing power

Table 5: Results of reducing power assay, showing concentrations of the drug, *i.e.*, standard/plant extract (leaf powder of *A. marmelos, C. dactylon*, *A. squamosa* and *H. rosa-sinensis*) (mg/ml) against the absorbance (taken at 700 nm)

Tube No.	Concentration of standard/plant extract (mg/ml)	Absorbance of standard at 700 nm	Absorbance of AME at 700 nm	Absorbance of CDE 700 nm	Absorbance of ASE 700 nm	Absorbance of HRS 700 nm
1.	2	0.42 ± 0.02	0.30 ± 0.03	0.25 ± 0.04	0.40 ± 0.06	0.40 ± 0.04
2.	4	0.53 ± 0.06	0.35 ± 0.05	0.30 ± 0.03	0.45 ± 0.04	0.52 ± 0.04
3.	6	0.62 ± 0.05	0.42 ± 0.04	0.35 ± 0.07	0.53 ± 0.02	0.59 ± 0.06
4.	8	0.70 ± 0.03	0.49 ± 0.06	0.42 ± 0.05	0.59 ± 0.01	0.65 ± 0.02
5.	10	0.75 ± 0.01	0.56 ± 0.02	0.50 ± 0.01	0.65 ± 0.03	0.72 ± 0.05

*All the values are expressed as mean ± SD after three determinations.

3.2.5 Hydroxyl radical scavenging assay (OH-scavenging)

Table 6: Results of OH-scavenging assay, showing concentrations of the drug, *i.e.*, standard/plant extract (leaf powder of *A. marmelos, C. dactylon*, *A. squamosa* and *H. rosa-sinensis*) (mg/ml) and the OH scavenged (% activity) shown by the drug, *i.e.*, standard/plant extracts

Tube No.	Concentration of plant extract (mg/ml)	OH scavenged (% activity) acid standard ascorbic	OH scavenged (% activity) AME	OH scavenged (% activity) CDE	OH scavenged (% activity) ASE	OH scavenged (% activity) HRS
1	10	69.44 ± 0.11	51.38 ± 0.22	45.83 ± 0.14	37.5 ± 0.10	52.98 ± 0.20
2	30	73.69 ± 0.10	56.94 ± 0.09	48.61 ± 0.21	44.44 ± 0.24	58.24 ± 0.12
3	50	77.77 ± 0.20	61.11 ± 0.17	52.78 ± 0.12	50.00 ± 0.20	62.10 ± 0.18
4	70	80.55 ± 0.22	62.50 ± 0.21	56.94 ± 0.34	54.17 ± 0.17	63.50 ± 0.19
5	90	83.33 ± 0.09	63.89 ± 0.11	61.11 ± 0.16	56.94 ± 0.11	64.89 ± 0.09

*All the values are expressed as mean ± SD after three determinations.

Table 7: Showing IC_{50} values (mg/ml of standard/plant extract standard/plant extract (leaf powder of *A. marmelos, C. dactylon*, *A. squamosa* and *H. rosa-sinensis*)

S. No.	Plant sample	IC_{50} (mg/ml of plant sample)
1	Standard	7.26 ± 0.10
2	AME	10.00 ± 0.12
3	CDE	36.60 ± 0.15
4	ASE	49.24 ± 0.16
5	HRS	8.90 ± 0.15

*All the values are expressed as mean ± SD after three determinations.

3.2.6 Nitric oxide radical scavenging assay (NO-scavenging)

Table 8: Results of NO-scavenging assay, showing concentrations of the drug, *i.e.*, standard/plant extract (leaf powder of *A. marmelos, C. dactylon*, *A. squamosa* and *H. rosa-sinensis*) (mg/ml) and the NO scavenged (% activity) shown by the drug, *i.e.*, standard ascorbic acid/plant extracts

Tube No.	Concentration of plant extract (mg/ml)	NO scavenged (% activity) standard ascorbic acid	NO scavenged (% activity) AME	NO scavenged (% activity) CDE	NO scavenged (% activity) ASE	NO scavenged (% activity) HRS
1	10	58.33 ± 0.12	40.00 ± 0.22	20.00 ± 0.23	21.67 ± 0.12	49.00 ± 0.20
2	30	63.33 ± 0.10	50.00 ± 0.18	33.33 ± 0.12	36.67 ± 0.09	63.33 ± 0.10
3	50	71.66 ± 0.14	60.00 ± 0.24	50.00 ± 0.24	53.33 ± 0.16	68.00 ± 0.26
4	70	76.66 ± 0.09	70.00 ± 0.16	58.33 ± 0.16	66.67 ± 0.11	74.23 ± 0.15
5	90	78.33 ± 0.08	73.33 ± 0.08	61.67 ± 0.08	70.00 ± 0.12	76.97 ± 0.07

*All the values are expressed as mean ± SD after three determinations.

Table 9: Showing IC_{50} values (mg/ml of standard/plant extract standard/ plant extract (leaf powder of *A. marmelos*, *C. dactylon*, *A. squamosa* and *H. rosa-sinensis*)

S. No.	Plant sample	IC_{50} (mg/ml of plant sample)
1	Standard	9.24 ± 0.10
2	AME	30 ± 0.18
3	CDE	50 ± 0.15
4	ASE	45.95 ± 0.13
5	HRS	18.45 ± 0.14

*All the values are expressed as mean ± SD after three determinations.

3.2.7 Superoxide anion radical scavenging (SO) activity

Table 10: Results of SO-scavenging assay, showing concentrations of the drug, *i.e.*, standard/plant extract (leaf powder of *A. marmelos, C. dactylon*, *A. squamosa* and *H. rosa-sinensis*) (mg/ml) and the SO scavenged (% activity) shown by the drug, *i.e.*, standard ascorbic acid/plant extract

Tube No.	Concentration of plant extract (mg/ml)	SO scavenged (% activity) atandard ascorbic acid	SO scavenged (% activity) AME	SO scavenged (% activity) CDE	SO scavenged (% activity) ASE	SO scavenged (% activity) HRS
1	10	72.41 ± 0.10	41.38 ± 0.10	15.52 ± 0.18	60.35 ± 0.13	61.47 ± 0.11
2	30	75.86 ± 0.11	44.83 ± 0.23	29.31 ± 0.21	68.97 ± 0.18	68.67 ± 0.19
3	50	79.31 ± 0.12	51.72 ± 0.25	43.10 ± 0.19	74.14 ± 0.19	77.44 ± 0.17
4	70	82.76 ± 0.17	55.17 ± 0.22	56.90 ± 0.23	81.03 ± 0.14	81.67 ± 0.15
5	90	87.93 ± 0.18	60.35 ± 0.17	70.69 ± 0.11	84.48 ± 0.15	85.80 ± 0.13

*All the values are expressed as mean ± SD after three determinations.

Table 11: Showing IC_{50} values (mg/ml of standard/plant extract standard /plant extract (leaf powder of *A. marmelos*, *C. dactylon*, *A. squamosa* and *H. rosa-sinensis*)

S. No.	Plant sample	IC_{50} (mg/ml of plant extract)
1	Standard	6.6 ± 0.14
2	AME	44.62 ± 0.18
3	CDE	60 ± 0.18
4	ASE	7.92 ± 0.16
5	HRS	6.9 ± 0.16

*All the values are expressed as mean ± SD after three determinations.

4. Discussion

4.1 Phytochemical screening

The presence of phenol and alkaloids in acetone and methyl alcohol extracts of *A. marmelos* was reported by Ulahannan *et al.* (2008). Flavonoids were detected by them only in extract of the plant in methyl alcohol and terpenes were not detected in any of the tested extracts (Ulahannan *et al.*, 2011). However, in our study, phenols were present in the ethyl alcoholic and aqueous extracts of the leaves of *A. marmelos*. We found alkaloids to be present in all the tested polar and non-polar extracts, *viz.*, petroleum ether, chloroform, acetone, ethyl acetate, ethanol, methanol and water. Flavonoids were detected in both ethanolic and methanolic extracts and terpenoids in chloroform, acetone, ethanolic and methanolic leaf extracts of the plant. Phytochemical analysis of the polar and non-polar extracts of *A. marmelos* was also done by Rajeshwari *et al.* (2011) and the absence of terpenoids in all the extracts was reported by them. Another study reported the presence of alkaloids, flavonoids, phenols, saponins, steroids and tannins in the dried leaves of *A. marmelos* (Veer and Singh, 2019).

Preliminary phytochemical investigation of *C. dactylon* was done by Siddiqui *et al.* (2009). The methanol and aqueous extracts of the leaves of *C. dactylon* were investigated by them for the presence of various phytochemicals and both the extracts showed presence of alkaloids and phenols. This observation reported by Siddiqui *et al.* (2009), correlates with the results of our study, which indicates presence of alkaloids in all the tested extracts including methanol and aqueous extracts and presence of phenols in ethanol, methanol and aqueous extracts of the leaves of *C. dactylon.* Brindha *et al.* (2018) observed the presence of alkaloids, flavonoids, phytosterols, tannins and phenols in alcoholic and aqueous extract of the *C. dactylon*.

The presence of alkaloids, coumarins, glycosides, terpenoids and steroids, saponins and tannins in the petroleum ether and ethanol extracts of *A. squamosa* leaves has been reported by Tuan *et al.* (1994). These results are in accordance with our observations, which indicate presence of alkaloids, glycosides, terpenoids and steroids, phenols, flavonoids and tannins in the leaf extract of *A. squamosa* in ethyl alcohol. However, the presence of saponins was noted by us in the aqueous extract than in the extract of the plant sample in ethyl alcohol. In our study, the presence of alkaloids, steroids and phytosterols was observed in the petroleum ether extract of *A. squamosa* leaves. Another study reported that the leaves and bark of *A. squamosa* contain a significant amount of alkaloid, flavonoids, phenolic, saponins and tannins (Marhatta *et al.*, 2019).

In our study, effective extraction of various phytochemicals was seen more in polar solvents like ethanol, methanol and water extracts than the non-polar solvent extracts of *H. rosa-sinensis.* Paul *et al.* (2016) also reported the presence of saponins, phenols, alkaloids, proteins/amino acids, tannins, flavonoids, carbohydrates/reducing sugars and resins in the aqueous extract of *H. rosa-sinensis* leaves. Udo *et al.* (2016) revealed the presence of tannins, combined anthraquinones, cardiac glycosides and alkaloids in the alcoholic extract of *H. rosa-sinensis* leaves.

4.2 *In vitro* antioxidant study

4.2.1 Total antioxidant activity (TAA)

From the Table 2, it is clear that the leaf extracts of *A. marmelos* and *A. squamosa* in methyl alcohol exhibited the value of 2.709 ± 0.08 mg of gallic acid equivalents (GAE)/g of the plant material and 3.833 ± 0.10 mg of gallic acid equivalents (GAE)/g of the plant material, respectively. The leaf extract of *C. dactylon* in methyl alcohol showed the value of 3.482 ± 0.12 mg of gallic acid equivalents (GAE)/g of the plant material. The results of the present study are in agreement with that Jananie *et al.* (2011) who have done analogous study earlier using hydroalcoholic extracts of *C. dactylon* and have reported that the hydroalcoholic extract of *C. dactylon* is rich in antioxidant components (Jananie *et al.*, 2011). The total antioxidant activity of the hydroalcoholic extract of *C. dactylon* reported by them in the equivalents of ascorbic acid as 172.39 mg/ g of extract.

The leaf extract of *H. rosa-sinensis* in methyl alcohol showed the value of 12.126 ± 0.22 mg of gallic acid equivalents (GAE)/g of the plant material. Our results are similar to the results of Nascimento *et al.* (2021) who reported that flavonoid and phenolic compounds are high in *H.rosa-sinensis*, also exhibiting greater total antioxidant activity.

4.2.2 Total phenolic content (TPC)

From the Table 3, it is clear that the leaf extract of *A. marmelos* in methyl alcohol showed the value of 4.402 ± 0.11 mg of gallic acid equivalents (GAE)/g of the plant material. However, Siddiqui *et al.* (2009) has reported the variation in the phenolic content of leaves of *A. marmelos* from 9.8367 ± 0.0235 mg/g to 1.7281 ± 0.049 mg/g. This minor difference from our results might be due to the variation in extraction method adapted and also due to different environmental conditions.

The methyl alcohol leaf extract of *A. squamosa* expressed the value of 5.693 ± 0.12 mg of gallic acid equivalents (GAE)/g of the plant material. Studies of Biva *et al.* (2009) indicated the highest total phenolic content of the leaves of *A. squamosa* was in the methyl alcohol soluble extract (283.16 ± 8.90 mg/g), followed by chloroform soluble extract (216.90 ± 4.48 mg/g) and then n-hexane (89.49 ± 2.48) (Biva *et al.*, 2009). However, in our present study, the total phenolic content of the leaves of *A. squamosa* was found to be 5.693 ± 0.12 mg of gallic acid equivalents (GAE)/g of the plant material.

The leaf extract of *C. dactylonin* methyl alcohol showed the value of 5.342 ± 0.09 mg of gallic acid equivalents (GAE)/g of the plant material. Jananie *et al.* (2011) have reported the hydroalcoholic extract of *C. dactylon* showed the total phenolic content of 4.029 mg/g (as tannic acid equivalents), which was very close to the value reported by us.

The leaf extract of *H.rosa-sinensis* in methyl alcohol showed the value of 26.32 ± 0.24 mg of gallic acid equivalents (GAE)/g of the plant material. Results of Ghaffar and Ibrahim (2012) showed the total phenolic content of 48.4 mg/g (as tannic acid equivalents), which was higher than the value reported by us.

4.2.3 Total flavonoid content (TFC)

The total flavonoid content of the methanolic leaf extract of *A. marmelos* as noted by us is 8.360 ± 0.10 mg of quercetin equivalents (QE)/g of the plant material (Table 4). The results of the present study are in agreement with that of Siddiqui *et al.* (2009) who reported the total flavonoid contents of the methanolic leaf extract of *A. marmelos* to vary between 8.248 ± 0.029 to 1.087 ± 0.002 mg/g.

The leaf extract of *A. squamosa* in methyl alcohol as noted by us is 5.693 ± 0.06 mg of quercetin equivalents (QE)/g of the plant material

(Table 4). However, the total flavonoid content reported earlier by Soni (2011) in the leaf extract of *A. squamosa* is 2.4 %.

The leaf extract of *C. dactylon* in methyl alcohol as noted by us is 8.661 $\pm$ 0.11 mg of quercetin equivalents (QE)/g of the plant material (Table 4). However, the value for the total flavonoid content of the ethanolic extract of *C. dactylon* reported by Jananie *et al.* (2011) is 0.17 mg/g of extract, which varies markedly from the value obtained by us.

The leaf extract of *H.rosa-sinensis* in methyl alcohol expressed the value of 17.58 $\pm$ 0.19 mg of gallic acid equivalents (GAE)/g of the plant material (Table 4). Results of Ghaffar and Ibrahim (2012) showed the total flavanoid content of 24.26 mg/g (as quercitin equivalents), which was close to our observations.

4.2.4 Reducing power

The reducing powers of the methanolic leaf extracts of *A. marmelos*, *A. squamosa*, *A. squamosa* and *H. rosa-sinensis* are shown in Table 5. For the measurements of the reductive ability, we investigated the Fe^{3+} to Fe^{2+} transformation in the presence of the plant extract using the method of Oyaizu (1986). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant (Mier *et al.,* 1995). The reducing power augmented with the increased concentration of the extract, indicating that tested plant extracts could behaving compounds which are electron donors and could react with free radicals to neutralize them and convert them into more stable products, thus inhibiting the radical chain reactions. All tested plant extracts showed significant activity when compared to the respective standards and the difference was statistically significant ($p < 0.01$) for all. Similar findings were reported by Jananie *et al.* (2011) for the ethanolic extract of *C. dactylon* with maximum scavenging activity as 67.69% at 1mg/ml concentration and the IC_{50} value as 632¼ g/ml.

Ghadir *et al*. (2011) reported that the *A. squamosa* leaf extracts showed different capacities for electron donation which was found to be proportionally related to the extract concentration, however, they found that the activities were inferior to that of BHT, used as reference.

4.2.5 Hydroxyl radical scavenging assay (OH-scavenging)

The inhibition showed by *A. marmelos, A. squamosa*, *C. dactylon* and *H. rosa-sinensis* was (63.89 $\pm$ 0.11%), (56.94 $\pm$ 0.11%), (61.11 $\pm$ 0.16%) and (64.89 $\pm$ 0.09%), respectively. The highest scavenging capacity on hydroxyl radicals was showed by the ascorbic acid (vitamin C) which was (83.33 $\pm$ 0.09%) at 90 mg/ml concentration.Vitamin C was used as a standard. The reduction ability is observed in a dose dependent manner, *i.e.*, increasing with increasing concentrations (Table 6).

The IC_{50} values of methanolic leaf extracts of *A. marmelos, A. squamosa*, *C. dactylon* and *H. rosa-sinensis* were (10 $\pm$ 0.12 mg/ml), (49.24 $\pm$ 0.16 mg/ml), (36.60 $\pm$ 0.15 mg/ml) and (8.90 $\pm$ 0.15 mg/ml), respectively. Vanitha *et al*. (2010) reported the alcoholic leaf extract of *A. squamosa* showed significant hydroxyl radicals scavenging activity at 500¼ g/ml concentration. However, our study indicated that the maximum scavenging potential on hydroxyl radicals was shown by the methanolic leaf extract of *A. squamosa* at 56.94 $\pm$ 0.11 % at the highest concentration tested (90 mg/ml).

In our study, the IC_{50} value of *H.rosa-sinensis* was found to be lowest (8.90 $\pm$ 0.15 mg/ml) among the plant extracts under study and was very close to the IC_{50} value of the ascorbic acid standard (7.26 $\pm$ 0.10 mg/ml), suggesting very high scavenging potential on hydroxyl radicals (Table 7).

4.2.6 Nitric oxide radical scavenging assay (NO-scavenging)

The results of the study are presented in Table 8 and the IC_{50} value (mg/ml of plant sample) is presented in Table 9. Table 8 shows the percentage inhibition of nitric oxide generation by the above mentioned plants and ascorbic acid, which was used as a standard.

In this study, the inhibitory effect of the crude methanolic extract of the leaves of *A. marmelos, A. squamosa*, *C. dactylon* and *H. rosa-sinensis* on nitric oxide generation was checked. Our study showed that all the plant leaf extracts under study effectively prevented the formation of peroxynitrate and can be used for the prevention of the adverse effects of metabolites of nitric oxide.

In the tested concentration ranging from 10-90 mg/ml in our study, we observed maximum scavenging activity (% inhibition) on nitric oxide radicals, when the concentrations of leaf extracts of the plants in methyl alcohol was around 90 mg/ml.

At the highest concentration tested (90 mg/ml), inhibition showed by *A. marmelos, A. squamosa*, *C. dactylon* and *H. rosa-sinensis* was (73.33 $\pm$ 0.08%), (70.00 $\pm$ 0.12%), (61.67$\pm$ 0.08%) and (76.97$\pm$ 0.07%), respectively. The maximum scavenging capacity on nitric oxide radicals was showed by ascorbic acid, which was used as a standard for *A. marmelos, A. squamosa*, *C. dactylon* and *H. rosa-sinensis* was (78.33 $\pm$ 0.08%),(78.33 $\pm$ 0.08%), (78.33 $\pm$ 0.08%) and (78.33 $\pm$ 0.08%), respectively, at 90 mg/ml concentration. The reduction ability increased with increasing concentrations, *i.e.*, dose dependent manner.

It was reported by Vanitha *et al*. (2010) that the alcoholic leaf extract of *A. marmelos* scavenges the NO radical more effectively than that of *A. squamosa* at 500 ¼ g/ml concentration. However, in our study, *A. squamosa* showed inhibition of 70.00 $\pm$ 0.12% at the concentration of (90 mg/ml), which was the highest concentration used. Baskar *et al*. (2007) had also earlier reported that the scavenging activity (% inhibition) on nitric oxide radicals by ethanolic extract of *A. squamosa* leaves as 68.03% at 500 ¼ g/ml concentration.

The concentration of *A. marmelos, A. squamosa*, *C. dactylon* and *H. rosa-sinensis* needed for 50% inhibition of nitric oxide radical (IC_{50} value) was found to be 30 $\pm$ 0.18 mg/ml; 45.95 $\pm$ 0.13 mg/ml; 50 $\pm$ 0.15 mg/ml and 18.45 $\pm$ 0.14 mg/ml, respectively, whereas (9.24 $\pm$ 0.10 mg/ml) was needed for ascorbic acid used as standard (Table 9).

It has been reported by Rastogi *et al*. (1996) that the maximum activity noted in *A. marmelos* could be due to the flavonoids present in them. Bael has been reported to contain aegelinine, cineole which possess antioxidative and free radical scavenging activity (Rastogi and Mehrotra, 1996).Vanitha *et al*. (2010) reported the alcoholic leaf extract of *A. marmelos* showed better activity with nitric oxide radical and inhibited the generation of anions. It was also reported by Vanitha *et al*. (2010) that the alcoholic leaf extract of *A. marmelos* scavenges the NO radical more effectively than that of *A. squamosa* at 500 ¼ g/ ml concentration. The results of our study were found to be in agreement with the findings of Vanitha *et al*. (2010) and indicated that the maximum scavenging activity (% inhibition) on nitric oxide radicals was shown by the methanolic leaf extract of *A. marmelos* which showed maximum inhibitory effect (73.33 $\pm$ 0.08%) inhibition.

The maximum scavenging activity of nitric oxide of the ethanolic extract of *C. dactylon* as reported by Jananie *et al*. (2011) was 71.28% at a concentration of 0.5 mg/ml of the extract and its IC_{50} value was 115 ¼ g/ml whereas, Bhalerao *et al*. (2011) reported the IC_{50} value of the ethanolic and petroleum ether extract of *C. dactylon* as (74.00 ± 2.14%) and (89.40 ± 2.14%), respectively, in the nitric oxide scavenging method.

4.2.7 Superoxide anion radical scavenging (SO) activity

The results of the study are presented in Table 10 and the IC_{50} values (mg/ml of plant extracts) is indicated in Table 11. Table 10, shows the percentage inhibition of superoxide anion radicals by the methanolic leaf extracts of *A. marmelos, A. squamosa*, *C. dactylon* and *H. rosa-sinensis* and the ascorbic acid (vitamin C) as a standard used.

In the present study, the superoxide anion radicals are derived in PMS NADH NBT system, superoxide radical reduces NBT to blue coloured formazan that is measured at 560 nm (Bhalerao *et al*., 2011) and the decrease in absorbance at 560 nm indicates the consumption of superoxide anion in the reaction mixture, thereby exhibiting a dose dependent increase in superoxide scavenging activity (Khanam *et al*., 2004).

We tested the superoxide anion radicals scavenging activities of methanolic leaf extracts of *A. marmelos, A. squamosa*, *C. dactylon* and *H. rosa-sinensis* in the concentration ranging from 10-90 mg/ml (Table 10). In the range tested, we observed that the highest scavenging activity (% inhibition) on superoxide anion radicals was found when the concentrations of leaf extract of the plant in methyl alcohol was about 90 mg/ml. At the highest concentration tested (90 mg/ml), the inhibition shown by *A. marmelos, A. squamosa*, *C. dactylon* and *H. rosa-sinensis* was (60.35 ± 0.17%), (84.48 ± 0.18%), (70.69 ± 0.11%) and (85.80 ± 0.13%), respectively. Ascorbic acid used as a standard in this assay showed the maximum superoxide anion radical scavenging capacity of 87.93 ± 0.18% at 90 mg/ml concentration. The reduction ability was observed in a dosedependent manner, *i.e.,* increasing with increasing concentrations.

The concentration of *A. marmelos, A. squamosa*, *C. dactylon* and *H. rosa-sinensis* needed for 50% inhibition of superoxide anion radical (IC_{50} value) was found to be (44.62 ± 0.18 mg/ml), (7.92 ± 0.16 mg/ ml), (60 ± 0.18 mg/ml) and (6.9 ± 0.16 mg/ml), respectively, whereas (6.6 ± 0.14 mg/ml) was needed for ascorbic acid used as standard (Table 11).

Vidhya and Devaraj (1999) reported that *A. marmelos* has high concentration of eugenol, tannins and phlobatannins. Hence, the high scavenging activity of *A. marmel os*observed could be due to eugenol which in high concentration and lipid peroxidation may be inhibited. Vanitha *et al*. (2010) reported that the alcoholic leaf extract of *A. marmelos* showed significant superoxide anion radical scavenging activity. They also reported that the alcoholic leaf extract of *A. marmelos* showed significant superoxide anion radical scavenging activity than *A. squamosa.* However, the results of our study indicated that the scavenging potential of superoxide anion radical shown by the methanolic leaf extract of *A. squamosa* has the maximum inhibitory effect of 84.48 ± 0.18% inhibition at the highest concentration tested (90 mg/ml). Ealier, Baskar *et al*. (2007) had also reported the scavenging activity (% inhibition) on superoxide anion radicals by ethanolic extract of *A. squamosa* leaves as 77.21% at 500 ¼ g/ml concentration.

The maximum superoxide anion radical scavenging activity of the ethanolic extract of *C. dactylon* as reported by Jananie *et. al*. (2011) was 93.33% at a concentration of 1.5 mg/ml of the extract and its IC_{50} value was reported to be 430.06 ¼ g/ml.

Overall, the results obtained in the present study indicated that the *A.marmelos*, *A. squamosa*, *C. dactylon* and *H. rosa-sinensis* showed comparatively good scavenging activity, *i.e.*, inhibition of hydroxyl radical, nitricoxide, superoxide anion scavenging and reducing power activities in comparison with the respective standards.

5. Conclusion

Thus, it can be concluded that, the leaf powders of the plants studied can prove to be easily accessible source of natural antioxidant, thus making them a good choice for its use as food supplement or in pharmaceutical industry. However, further work should be undertaken on the isolation and identification of the antioxidant components in the leaf powders of the mentioned plants as the exact molecules and elements responsible for the antioxidant activity of this plant leaf powder are currently unclear.

Acknowledgements

Authors acknowledge the enormous help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to publishers/editors/ authors of all those articles, journals and books used to review the literature for this article.

Conflicts of interest

The authors declare no conflicts of interest relevant to this article.

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Citation **Samidha M. Pawaskar and Ketan J. Ranade (2022). Phytochemical analysis and *in vitro* antioxidant studies of the leaf extracts of some Indian medicinal plants from western Maharashtra. Ann. Phytomed., 11(2):507-515. http://dx.doi.org/10.54085/ap.2022.11.2.62.**