ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 12, Issue 8, 2019

Online - 2455-3891
Print - 0974-2441

Research Article

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT POTENTIAL OF *ANACARDIUM OCCIDENTALE, ACHYRANTHES ASPERA,* AND *AEGLE MARMELOS*

BRIJYOG[1,2]*, LALITESHWAR PRATAP SINGH[1], SUSHIL KUMAR[2], SHWETA VERMA[2]

[1]Department of Pharmacy, Institute of Pharmacy, Harish Chandra P.G. College Varanasi-221002, Uttar Pradesh, India. [2]Department of Pharmacy, Faculty of Pharmacy, IFTM University, Moradabad, Uttar Pradesh, India. Email: r.brijyog@rediffmail.com

Received: 24 May 2019, Revised and Accepted: 25 June 2019

ABSTRACT

Objective: *Anacardium occidentale*, *Achyranthes aspera,* and *Aegle marmelos* are the common plants found in North and South India. The present study identifies the active phytochemicals and antioxidant properties in various extracts derived from dried bark, whole aerial parts, and leaves of the above plants.

Materials and Methods: Phytochemical screening included extracts of pet ether, hydroalcoholic, and aqueous. The antioxidant activity was determined by measuring total phenolic contents (TPC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (RSA), and total flavonoid content (TFC).

Results: The phytochemical screening of *A. occidentale, A. aspera,* and *A. marmelos of* dried bark, whole aerial parts, and leaves revealed the presence of flavonoids, phenols, tannins, and proteins. It also contains alkaloids and glycosides. Hydroalcoholic extract of *A. occidentale, A. aspera,* and *A. marmelos* showed highest TPC 0.125 mg/g, 0.256 mg/g, and 0.254 mg/g, respectively. TFCs 0.094 mg/g, 0.145 mg/g, and 0.121 mg/g and highest DPPH RSA with the half maximal inhibitory concentration of 125 µg/mL, 105.58 µg/mL, and 98.89 µg/mL compare to the standard ascorbic acid of 21.65 µg/mL.

Conclusion: This study showed that the hydroalcoholic extract of *A. occidentale, A. aspera,* and *A. marmelos* is potential source of natural antioxidants.

Keywords: Antioxidant activity, Flavonoids, Phenols, *Anacardium occidentale, Achyranthes aspera, Aegle marmelos.*

 DOI: http://dx.doi.org/10.22159/ajpcr.2019.v12i8.34147

INTRODUCTION

The matter of proper identification and appropriate quality that is lack of adulteration, sophistication, or substitution is an extremely important one in the field of herbal medicine. Nowadays, widely used herbs were once the subject of official monographs of the United States Pharmacopeia and the National Formulary. These monographs established legal standard of identity and subject to the limitations of the methods, period, and quality of the herbal drugs.

The basis of standardized extract gives a solid platform for scientific validation of herbals. Plant materials isolated from them show a substantial proportion of world drug market and globally recognized the guidelines for the assessment of quality are necessary. The quality of medicinal plant for therapeutic purpose must be very high. However, it is very difficult to assay for specific chemical entity provided that the bioactive ingredient is unknown [1,2].

Plants are the potential source of natural antioxidants. Natural antioxidants or phytochemical antioxidants are the secondary metabolites of plants [3]. Plant-derived antioxidants have received greater attention since they act as radical scavengers. To the best of our knowledge, no *in vitro* antioxidant assay of *Anacardium occidentale, Achyranthes aspera,* and *Aegle marmelos* has been reported. Therefore, the study was aimed to determine total phenolic and flavonoid contents and evaluate the antioxidant activity of *A. occidentale, A. aspera,* and *A. marmelos.*

MATERIALS AND METHODS

Chemicals and reagents

All the chemicals used were of analytical grade. The chemicals were purchased from Sigma-Aldrich, Bangalore, Global Chemie (India), and Qualikems (India). Sterile distilled water was used, whenever required for the experiment.

Plant material

A. occidentale, A. aspera, and *A. marmelos* were collected in August 2016, from the Vindhya Herbal Garden, Bhopal, Madhya Pradesh. The plant material was authenticated and confirmed by Dr. Suman Trivedi, Professor, Department of Botany, Government Maharani Laxmi Bai Girls Postgraduate College, Bhopal, and voucher specimen was deposited as herbarium at the Department of Botany. The collected plant materials were air-dried in dark room at 20°C. Dried plant parts were cut into small pieces and stored in tight seal light-resistant containers until needed.

Preparation of plant extracts

The preparation of plant extracts was done as per standard protocol. Transferred 10 g of plant extract to dark color flask and mixed 200 mL of solvents petroleum ether, hydroalcoholic, and aqueous, extract was stored at room temperature. After 24 h, infusions were filtered through Whatman filter paper and residue was reextracted with equal volume of solvents. After 48 h, the process was repeated. Combined supernatants were evaporated to dryness under vacuum at 40°C using rotary evaporator. The obtained extracts were kept in sterile sample tubes and stored in a refrigerator at 4°C [4].

Total phenolic contents (TPC) determination

Folin–Ciocalteu method was used for the determination of the TPC of the plant extracts using gallic acid as an internal standard with slight modification as previously reported [5]. About 1 mL of the extract (1 mg/mL) was mixed with 9 mL of distilled water in a 25 mL volumetric flask. Two and half milliliter (2.5 mL) of a 10-fold dilute Folin–Ciocalteu phenol reagent (1:10) was added. After 5 min, 10 mL of 7% Na_2CO_3 solution was added to the mixture and made up to the mark with distilled water. The mixture was incubated for 90 min at room temperature. A set of standard solutions of gallic acid was prepared in the same manner as described for the extracts. The absorbance of the

extracts and standard solutions were measured against the reagent blank at 750 nm with an ultraviolet (UV)/visible spectrophotometer (UV-1800, Shimadzu, Japan). The TPC was determined from the calibration curve and expressed as milligram of gallic acid equivalent (GAE) per gram of the extracts [6]. The determination of the total phenolic in the extract was carried out in triplicate.

Determination of total flavonoid content (TFC)

Aluminum-chloride colorimetric assay was used to determine the TFC in the extracts as previously reported [7]. About 1 mL of the extract (1 mg/mL) was mixed with 4 mL of distilled water in a 10 mL volumetric flask. About 0.30 mL of 5% sodium nitrite was added to the flask. After 5 min, 0.30 mL of 10% $AlCl_3$ solution was added to the mixture, followed by addition of 2 mL of 1 M NaOH after another 5 min and diluted to the mark with distilled water. A set of standard solutions of quercetin was prepared in the same manner as described for the extracts. The absorbances of the extracts and standard solutions were measured against the reagent blank at 510 nm with a UV/visible spectrophotometer. The TFC was determined from the calibration curve and expressed as milligram of quercetin equivalent (QE) per gram of extracts [8]. The determinations of total flavonoid in the extracts and standards were carried out in triplicates.

Qualitative phytochemical analysis of the extracts

Phytochemical analysis of the plant extracts was done to determine the presence of flavonoids, phenol, protein, carbohydrate, alkaloid, and glycoside according to standard methods [9].

Determination of antioxidant capacity

The antioxidant capacities of the plants of different extract were studied by the evaluation of the free radical-scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) by the modified method [10]. Various concentrations (20–100 µg/ml) of plant extracts (0.3 mL) were mixed with 2.7 mL of appropriate solvent (methanol) containing DPPH radical (0.1 mM). The radical stock solution was prepared fresh daily. Ascorbic acid was used as standard in 20–100 µg/mL solution. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 60 min. The decrease in absorbance of the resulting solutions was monitored at 517 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration, using the equation: % RSA=([A_{DPPH}−As/A_{DPPH}]×100) [11].

Where, As=Absorbance of the solution when the sample extracts were added at a particular level and A_{DPPH}=Absorbance of DPPH radical solution. All determinations were performed in triplicate. The half maximal inhibitory concentration (IC_{50}) was calculated as the amount of antioxidants present in the sample necessary to reduce the initial DPPH concentration by 50%.

Statistical analysis

All experimental measurements were carried out in triplicate and are expressed as average of three analyses ± standard deviation. The direction and magnitude of correlation between variables was done using analysis of variance (ANOVA) and quantified by the correlation factor.

RESULTS

A small portion of the dried extracts was subjected to the phytochemical test identification of carbohydrates, alkaloids, steroids, glycosides, tannins, saponins, flavonoids, proteins, and starch separately for extracts of all samples. Small amount of each extract was suitably centrifuged into the sterile distilled water to make the concentration of 1 mg/ml. The outcomes of the results are discussed in Tables 1-3.

From the results obtained, it is clear that *A. occidentale, A. marmelos,* and *A. aspera* hydroalcoholic extract shows the presence of protein, phenol, amino acid, steroid, and flavonoids. Flavonoids and phenol are the phytochemicals that are present in all the hydroalcoholic extracts. Hydroalcoholic extracts of *A. occidentale, A. marmelos,* and *A. aspera* were found highly rich in flavonoids and phenols.

TPC estimation

Phenolic compounds are a class of antioxidant agents which act as free radical terminators and their bioactivities may be related to their abilities to chelate metals, inhibit lipoxygenase, and scavenge free radicals. The amount of total phenol was determined with the Folin–Ciocalteu reagent.

The content of TPC was expressed as mg/g of GAE of dry extract sample using the equation obtained from the calibration curve: Y=0.038X+0.001, R^2=0.997, where X is the absorbance and Y is the GAE.

Gallic acid was used as a standard compound and the total phenols were expressed as mg/g GAE using the standard curve equation: Y=0.038X+0.001, R^2=0.997, where X is absorbance at 750 nm and

Table 1: Phytochemical constituents of *Aegle marmelos*. " + " indicates, presence; and " – " indicates, absent

Extracts	Alkaloids	Glycosides	Phenols/ tannins	Flavonoids	Saponins	Carbohydrates	Fats and oil	Steroids	Proteins
Hydroalcoholic	+	+	+	+	–	+	–	+	+
Aqueous	–	+	+	+	–	+	–	–	+
Pet ether	–	–	–	–	–	–	+	–	–

Table 2: Phytochemical constituents of *Achyranthes aspera.* " + " indicates, presence; and " – " indicates, absent

Extracts	Alkaloids	Glycosides	Phenols/ tannins	Flavonoids	Saponins	Carbohydrates	Fats and oil	Steroids	Proteins
Hydroalcoholic	+	+	+	+	+	+	–	+	+
Aqueous	–	+	+	+	+	+	–	–	–
Pet ether	–	–	–	–	–	–	+	–	–

Table 3: Phytochemical constituents of *Anacardium occidentale.* " + " indicates, presence; and " – " indicates, absent

Extracts	Alkaloids	Glycosides	Phenols/ tannins	Flavonoids	Saponins	Carbohydrates	Fats and oil	Steroids	Proteins
Hydroalcoholic	+	+	+	+	–	+	–	+	+
Aqueous	+	–	+	+	–	+	–	+	+
Pet ether	–	–	–	–	–	–	+	–	–

Y is TPC in the extracts of *A. occidentale, A. marmelos,* and *A. aspera* expressed in mg/g. Table 4 shows the variation of mean absorbance with concentration of gallic acid and shows the calibration curve of standard gallic acid. The contents of total phenols that were measured by Folin-Ciocalteu reagent in terms of GAE.

TFC estimation

TFC was calculated as QE (mg/g) using the equation based on the calibration curve: Y=0.041X+0.022, R^2=0.995, where X is the absorbance and Y is the QE. Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phytoconstituents.

The amount of total flavonoids was determined with the quercetin. Quercetin was used as a standard compound and the total flavonoids were expressed as mg/g QE using the standard curve equation: Y=0.041X+0.022, R^2=0.995, where Y is absorbance at 510 nm and X is TFC in the extracts of *A. occidentale, A. marmelos,* and *A. aspera* expressed in mg/g. Table 5 shows the variation of mean absorbance value with different concentration of quercetin reagent and shows the calibration curve of quercetin. The contents of total flavonoids were measured by $AlCl_3$ reagent in terms of QE.

The results obtained from the present study showed that the hydroalcoholic extracts of *A. occidentale, A. marmelos,* and *A. aspera* which contains highest amount of flavonoids and phenolic compounds, thus can be used to explore new drugs.

Antioxidant activity of hydroalcoholic extract

DPPH scavenging activity is being used by various researchers. It is very easy and reliable parameter for screening the *in vitro* antioxidant activity of plant extracts. DPPH is a stable free radical and accepts an electron to become a stable diamagnetic molecule. The absorption maximum of a stable DPPH radical in methanol was at 517 nm in UV spectrophotometer. It was observed that with the increase of concentration, there is decrease of absorbance value. The decrease in absorbance of DPPH radical caused by antioxidants, due to the reaction between antioxidants molecules and radical, progresses, which results in the scavenging of the radical by electron donation.

Table 4: Preparation of gallic acid calibration curve

S. No.	Concentration (µg/ml)	Absorbance (Mean) λ max=750 nm
1	1	0.041
2	2	0.073
3	3	0.115
4	4	0.154
5	5	0.210
6	6	0.231
7	7	0.273
8	8	0.301
9	9	0.342
10	10	0.389

Table 5: Preparation of quercetin calibration curve

S. No.	Concentration (µg/mL)	Absorbance (Mean) λ max=510 nm
1	1	0.071
2	2	0.115
3	3	0.155
4	4	0.195
5	5	0.235
6	6	0.275
7	7	0.312
8	8	0.351
9	9	0.391
10	10	0.438

The percentage of different concentration of standard ascorbic acid and *A. occidentale*, *A. marmelos,* and *A. aspera* is shown in Table 9.

IC_{50} for standard ascorbic acid was found to be 21.65 µg/ml and for hydroalcoholic extract of *A. occidentale*, *A. marmelos,* and *A. aspera* was found to be 125 µg/mL, 98.89 µg/mL, and 105.58 µg/mL, respectively. Thus, the antioxidant activity of sample was less than that of standard ascorbic acid.

Results are given in mean of triplicate. *A. aspera* extracts have high flavonoid/phenolics ratio, indicating that the extracts have high flavonoid content. The high antioxidant activity with these extracts due to the presence of phenol and flavonoid which are known to inhibit free radicals. The observed antioxidant activity could be attributed to these metabolites [12].

Free radicals are the cause for several major disorders. Hence, evaluation of antioxidant activity in plants could result in the discovery of natural antioxidants with pharmacological and food value. Therefore, these observations can be used in pharmaceutical to explore new drugs [Tables 6-8].

DISCUSSION

Phytochemical screening is done for analyzing secondary metabolites, which are responsible for curing ailments. Phytochemical screening of the extracts was investigated according to the standard procedure. The petroleum ether, hydroalcoholic, and aqueous extract of bark of *A. occidentale* and leaves of *A. marmelos* and whole aerial plant material of *A. aspera* were investigated to preliminary phytochemical screening for the presence of various phytoconstituents, i.e. alkaloids, terpenoids, steroids, flavonoids, carbohydrates, proteins, amino acids, tannins, and phenolic compounds present in them.

The results obtained it is clear that all selected plant extracts show the presence of alkaloids, phenols, and flavonoids, in petroleum ether, extract shows the presence of only fats and oil. Hydroalcoholic extract of *A. occidentale, A. marmelos, and A. aspera* shows the presence of alkaloids, glycoside, phenols, and flavonoids.

Quantitative analysis is an important tool for the determination of the quantity of phytoconstituents present in plant extracts. For this, TPC and TFC are determined. The hydroalcoholic extract obtained from bark of *A. occidentale*, leaves of *A. marmelos,* and whole aerial plant

Table 6: Total phenolic and flavonoids content of *Anacardium occidentale*

S. No.	Plant extracts	Total phenol (mg/g)	Total flavonoids (mg/g)
1	Hydroalcoholic	0.125	0.094
2	Aqueous	0.048	0.075

Table 7: Total phenolic and flavonoids content of *Aegle marmelos*

S. No.	Plant extracts	Total phenol (mg/g)	Total flavonoids (mg/g)
1	Hydro alcoholic	0.254	0.121
2	Aqueous	0.121	0.095

Table 8: Total phenolic and flavonoids content of *Achyranthes aspera*

S. No.	Plant extracts	Total phenol (mg/g)	Total flavonoids (mg/g)
1	Hydro alcoholic	0.256	0.145
2	Aqueous	0.110	0.075

Table 9: Result of *in vitro* free RSA

Concentration (µg/mL)	Ascorbic acid	Hydroalcoholic extracts		
	% inhibition	*Anacardium occidentale*	*Aegle marmelos*	*Achyranthes aspera*
20	92.197	58.996	78.184	87.579
40	92.914	63.535	79.777	90.047
60	93.789	66.321	82.882	91.082
80	96.417	71.815	84.235	92.436
100	97.452	75.00	87.579	94.028

Absorbance of control (Ao)=1.101

material of *A. aspera* is subjected to estimate the presence of TPC and TFC by standard procedure.

Phenolics play an important role in plant development, particularly in lignin and pigment biosynthesis. They provide structural integrity and scaffolding support to plants. In this study, the higher amount of TPC was recorded in the hydroalcoholic extract of *A. occidentale, A. marmelos,* and *A. aspera* (0.125 mg/g, 0.254 mg/g, and 0.256 mg/g) followed by aqueous extract (0.048 mg/g, 0.121 mg/g, and 0.110 mg/g), respectively [13].

Flavonoids play an important role in the protection of plants against plant-feeding insects and herbivores. Their presence can alter the palatability of the plants and reduce their nutritive value, decrease digestibility, or even act as toxins in few cases. The present work shows maximum TFC in the hydroalcoholic extract of *A. occidentale, A. marmelos,* and *A. aspera* (0.094 mg/g, 0.121 mg/g, and 0.145 mg/g) followed by aqueous extract (0.075 mg/g, 0.095 mg/g, and 0.082 mg/g), respectively.

Antioxidant plays a central role in defusing free radical species which are formed from various biochemical reactions in normal system. These free radicals are the main culprits in lipid peroxidation. The production of reactive oxygen species and free radicals occurs in various diseases, which directly/indirectly activates phagocytic cells. The synthetic medicines are known to produce severe side effects in the body. Phytodrug having antioxidant property is non-toxic or may have minimum side effects than synthetic compounds. The hydroalcoholic extract of *A. occidentale, A. marmelos,* and *Achyranthes* shows the IC_{50} value of 125 µg/ml, 98.89 µg/ml, and 105.58 µg/ml, respectively, and IC_{50} value for standard ascorbic acid was found to be 21.65 µg/ml. The antioxidant activity of sample was less than that of standard ascorbic acid [14].

CONCLUSION

From our studies, the extracts of medicinal plants were investigated for their total antioxidant capacity; the presence of high phenolics and flavonoids content. The high contents of phenolic compounds indicated that these compounds contribute to the strong antioxidant activity. Further studies of this plant species should be directed to carry out *in vivo* studies of its medicinal active components to prepare natural pharmaceutical products of high value.

AUTHORS' CONTRIBUTIONS

Sushil Kumar supervised the work; Brijyog and L. P. Singh conducted the experiments and monitored the work progress. Brijyog and Shweta Verma drafted the manuscript for publication. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest, financial, or otherwise.

REFERENCES

1. Singh PS, Bal M, Mukhtar MH, Shah G. Standardization and pharmacological investigation on leaves of *Ficus bengalensis*. Int J Res Pharm Chem 2011;1:891-903.
2. Robbers JE, Tyler VE. Tyler's Herbs of Choice: The Therapeutic use of Phytomedicinals. 3rd ed. New York: Haworth Press Inc.; 1999. p. 287-95.
3. Mukherjee PK. Quality Control of Herbal Drugs, an Approach to Evaluation of Botanicals. 1st ed. New Delhi: Business Horizon; 2002. p. 112-25.
4. Kujur RS, Singh V, Ram M, Yadava HN, Singh KK, Kumari S, *et al.* Antidiabetic activity and phytochemical screening of crude extract of *Stevia rebaudiana* in alloxan-induced diabetic rats. Pharmacogn Res 2010;2:258-63.
5. Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. BMC Complement Altern Med 2012;12:221.
6. Mythili K, Reddy CU, Chamundeeswari D, Manna PK. Determination of total phenol, alkaloid, flavonoid and tannin in different extracts of *Calanthe triplicat*a. J Pharmacogn Phytochem 2014;2:40-4.
7. Biju J, Sulaiman CT, Satheesh G, Reddy VR. Total phenolics and flavonoids in selected medicinal plants from Kerala. Int J Pharm Pharm Sci 2014;6:406-8.
8. Kostic DA, Dimitrijevic DS, Mitic SS, Mitic MN, Stojanovic GS, Zivanovic AV. Phenolic content and antioxidant activities of fruit extracts of *Morus nigra* L (*Moraceae*) from Southeast Serbia. Trop J Pharm Res 2013;12:105-10.
9. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Vol. 2. New York: Chapman and Hall; 1984. p. 288-95.
10. Braca A, Sortino C, Politi M, Morelli I, Mendez J. Antioxidant activity of flavonoids from *Licania licaniae* flora. J Ethnopharmacol 2002;79:379-81.
11. Helen LR, Jyothilakshmi M, Latha M. Antioxidant potential and antiproliferative effect on HCT-15cells. Int J Pharm Sci 2015;17:170-5.
12. Ahmad M, Shah AS, Khan RA, Khan FU, Khan NA, Shah MS, *et al*. Antioxidant and antibacterial activity of crude methanolic extract of *Euphorbia prostrata* collected from district Bannu (Pakistan). Afr J Pharm Pharmacol 2011;5:1175-8.
13. Ashalatha KS, Reddy AR, Raveesha HR. Evaluation of phytochemical compounds and antimicrobial activities of *Hemidesmus indicus*. Asian J Pharm Clin Res 2019;12:102-6.
14. Akila B, Manikandaselvi S. *In vitro* antioxidant and anticancer efficacies of ethanolic fruit extract of *Ziziphus jujube* mill. Asian J Pharm Clin Res2019;12:160-5.