ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

Pharmacognostical Standardisation of *Ailanthus Excelsa* Leaves and *Randia Dumetorum* Fruit Along with Antioxidant Activity and Free Radical Scavenging Capacity of Its Fractions

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Article History:

Received on: 21 Jul 2020
Revised on: 28 Aug 2020
Accepted on: 07 Sep 2020

Keywords:

Standardisation, Antioxidant Activity, Ailanthus Excelsa, Randia Dumetorum

ABSTRACT

The world is observing an unprecedented development in the usage of herbal product at national as well as international levels. This requires the improvement of current and aimed standards for estimating the quality, safety and efficacy of these drugs. The leaves of *Ailanthus excelsa* and the fruits of *Randia Dumetorum* are medicinal plants that are used for many diseases around the world. We then collected the flavonoids and saponin fraction extracted from the leaves of Ailanthus excelsa and the fruits of *Randia dumetorum*. To determine the reliability, quality and purity of these particles, we provide a crucial pharmacological profile along with the antioxidant activity. Pharmacological studies, such as morphological, physicochemical, TLC, and phytochemical analysis of all fractions containing total phenol and flavonoids, were performed according to specific methods. DPPH tests estimated the antioxidant action of all fractions, Hydrogen peroxide scavenging assay, and reducing power assay method. Previous phytochemical studies discovered the occurrence of saponins, flavonoids, tannins, and especially phenolic chemicals. All fractions have antioxidant effects, depending on the existence of a phenolic compound. The above parameters are vital to establishing pharmacological rules for the authentication of Ailanthus excelsa leaves and *Randia Dumetorum* fruits.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v11iSPL4.4268

Production and Hosted by

IJRPS | www.ijrps.com

INTRODUCTION

Presently there is an enormous deal of awareness in herbal medicine today. This interest is mainly because medicinal plants do not have adverse effects. A quarter of the world's population or 1.42 trillion people are not expected to rely on traditional medicines to treat various diseases (Kadam *et al.*, 2012). Traditional medical systems such as Ayurveda play an essential role in today's health field, especially in the treatment of malignant diseases (Shivatare *et al.*, 2014). However, the most significant barriers to the use of alternative drugs in industrialised countries are the lack of documentation and strict quality control. Research on traditional medicines needs to be documented. In this

context, it is vital to try to standardise the plant substance that will be utilised as a remedy (Modi *et al.*, 2010; Shruthi *et al.*, 2010). Normalisation can be done through pharmacological and phytochemical studies. These studies help to define and standardise plant substance. Appropriate detection with a quality guarantee of preliminary materials is essential requirements for the quality of phytotherapy reproduction, which contributes to improving its protection and usefulness (Sharma *et al.*, 2015; Kadam *et al.*, 2011).

Ailanthus excelsa (family- *Simaroubaceae)* is called "Mahanimba" as it resembles a neem plant (*Azadirachta indica*). The statement *Ailanthus* is resulting as of Ailanthus, and it means procession plant (Lavhale and Mishra, 2007). The name of one of the species of Moluccas, in Latin, means high excelsa. Recent research has shown that it contains six flavonoids secluded from the leaves of *A. excelsa* (Loizzo *et al.*, 2007). The bark of the leaves and the alcohol extracted from the stem show excellent anti-implantation activity and early abortion (Dhanasekaran *et al.*, 1993). *Randia Dumetorum* (*Xeromphis Spinosa*) belonging to family *Rubiaceae* is a vital medicine and is called deciduous (Movalia and Gajera, 2009).

A review of the literature shows that the results are bitter and sweet; warming, aphrodisiac, emetic, vaginal, carminative, antipyretic; It treats abscesses, ulcers, inflammation, wounds, tumours, skin diseases and has an antibacterial effect (Satpute *et al.*, 2012). Many experts believe that fruit fibres have anthelmintic properties and are also used in abortion as a folk medicine (Satpute *et al.*, 2009). Recently, the antioxidant properties of their components, usually polyphenol compounds, have been attributed to the function of medicinal plants in the manage or prevention of the disease. The intake of natural antioxidants while minimising the risk of cancer, diabetes, cardiovascular problems, and other age-related diseases (Subba and Mandal, 2015).

No information has been provided on pharmacological studies on the fruiting bodies of the leaves of Ailanthus excelsa and Randia Dumetorum. No data are provided on antioxidant activity. Therefore, this research focused on the standardisation of two plant fractions, measuring several aspects: morphology, physicochemistry, and TLC analysis. The content of qualitative and quantitative phytochemicals was also combined with antioxidant activities to investigate the presence of phytoactive ingredients.

MATERIALS AND METHODS

Reagent and Chemicals

The entire reagents and chemicals required for the evaluation of pharmacognostic, phytochemical screening, TLC and antioxidant activity were analytical grade obtained from J.T. Baker, Merck and Rankem.

Procurement, authentication and extraction of sample

The leaves of plant *Ailanthus excelsa* and Fruit of *Randia Dumetorum* were collected from fields of Bhopal (Madhya Pradesh) and validated by Safia College of Science, Bhopal. (Madhya Pradesh) were specified the specimen number 157/Bot/Safia/2010 (*Ailanthus excelsa*) and 158/Bot/Safia/2010 (*Randia Dumetorum*).

The leaves of plant *Ailanthus excelsa* and Fruit of *Randia Dumetorum* were clean and dehydrated below the shade. The desiccated samples were then ground into powdered form. All parts of the plant were crushed and extracted with water, using the method of decoration. This aqueous extract was then filtered, and alcohol (ethanol) was slowly added to this extracted liquid water to make polysaccharides. The solution was then filtered, and the filtered evaporated 1/4 of the total volume.

The same amount of ethyl acetate was then added using a separatory funnel to obtain a separate fraction of constituents of the roots in ethyl acetate. The ethyl acetate extract was acidified with 0.1 N HCl to increase the amount of the extract. The ethyl acetate fraction was evaporated to give the residue; it was then dissolved in methanol and evaporated to give a crystalline powder. Finally, the obtained powder was also analysed to determine the presence of phytoactive ingredients. In this study, it was seen that Shinoda responded positively to the flavonoid test. Besides, TLC confirmed the positivity of flavonoids in the appropriate solvent system (EAFW).

Same plant materials were used for getting saponins rich fraction. Pulverised plant materials were treated with ethanol: water (70:30) for maceration till seven days after defatting with petroleum ether (40:60). The blend was agitated at a normal interval in this stage. Obtained extract after filtration through muslin cloth followed by filter paper was concentrated using rotary vacuum evaporator (40°C), taking precaution that extract does not get powdered.

The concentrated extract was further treated with n-butanol to get an n-butanol soluble fraction. N-butanol soluble fraction was further treated with

chilled diethyl ether. After treating with chilled diethyl ether, the precipitate was formed. This mixture with precipitate was kept at -20°C for 24 hrs. Precipitates were further separated by centrifugation. This precipitate was further dissolved in methanol and methanol was evaporated slowly, to get crystalline powder.

Pharmacognostic Study

Macroscopical characters

External features, dimensions and organoleptic properties of leaves and fruits were studied.

Physicochemical Evaluation

The physicochemical properties of the component were evaluated to estimate the superiority and purity of the powder form. On physical evaluation, they were ash value viz., total ash value with acid insoluble ash value and water-soluble ash value were evaluated. The value of the residue indicates that the drug contains inorganic salts. Values for the extraction of water and soluble alcohol were established. The information obtained from these tests is useful for standardising and maintaining quality standards. These chemical-physical constants are determined according to the procedures mentioned under WHO guidelines (Ibrahim *et al.*, 2012; Baravalia *et al.*, 2011).

Phytochemical Investigations

The leaves of the fruits of *Ailanthus excelsa* and *Randia Dumetorum* were subjected to phytochemical studies before extraction. A preliminary phytochemical test was performed to confirm that the occurrence of different pharmacologically active phytochemicals in the crude powered drug. The tests were performed by a regular procedure (Kokate, 1994; Khandelwal, 2005).

TLC characterisation

TLC is generally utilised for the quick investigation of drugs and drug preparations. Fractions were put forwarded for the characterisation through thin layer chromatography (TLC; silica gel 60 F254, Merck). Chromatograms are estimated beneath U.V. light at 254 and 365 nm to identify the existence of flavonoids plus saponin, respectively. To verify the existence of flavonoids, TLC is furthermore sprayed by an Ammonia vapour. Saponin was identified by Anisaldehyde-sulphuric acid reagent along with Vanillin-phosphoric acid reagent.

Total phenolics Content

The Folin-Ciocalteu technique was applied to find out the entire phenolic content of the plant extract. Gallic acid is utilised as the standard to compare with fractions, and whole phenolic acid was articulated as mg / g gallic acid equivalent (GAE). 10, 20, 30, 40 and 50 (μg / ml) gallic acid concentrations be made in methanol. Sample of 1 mg / mL of plant extract in methanol was prepared, and 0.5 ml of every above-prepared sample was added for analysis and diluted with 2.5ml of a Ciocalteu-Folin reagent (10 times dilute) and mixed with 2 ml of 7.5 per cent sodium carbonate. The tubes are covered with paraffin and set aside at room temperature for 30 mins previous to the absorbance was read at 760 nm spectrometrically. All determination is finished three times. Folin-Ciocalteu reagent is responsive to reducing agents, as well as polyphenols. After the reaction, it turns blue. This blue colour was measured spectrophotometrically (Chang *et al.*, 2002).

Total flavonoid content

The colourimetric technique of aluminium chloride evaluated the total flavonoids contained inside the plant extract. Briefly, part of the sample was diluted (1 mg / ml) or standard solution (10, 20, 30, 40 and 50 μg / ml) to 75 μl. A solution of NaNO2 was added, and 0.15 ml of A1Cl3 was mixed 6 min earlier. Following 5 min, 1/2 ml of NaOH be added. The last amount was changed to 2.5 ml with purified water and mixed well.

The absorption of the combination is set at 510 nm compared to the similar combination, without the addition of the sample, as a blank. The entire flavonoid content was articulated as mg / g dry weight (mg / g D.W.) using the normal calibration curve. Every sample is scrutinised three times (Villaño *et al.*, 2007).

Studies of Antioxidant Activities

In vitro antioxidant activity of the different fractions at different concentration (Table 1) was studied by three procedures: with free radical scavenging by DPPH, Hydrogen peroxide assay and reducing power assay procedures.

Sample preparation

AEFF: *Ailanthus excelsa* Flavonoid Fraction

AESF: *Ailanthus excelsa* Saponin Fraction

RDFF: *Randia Dumetorum* Flavonoid Fraction

RDSF: *Randia Dumetorum* Saponin Fraction

DPPH free radical scavenging activity

To measure the DPPH radical scavenging activity, Barros *et al.* (2007) was carried out to the method. The drop of DPPH radicals was measured by determining the absorption at 517 nm. The radical scavanging activity was measured as a percentage of DPPH strains by the following equation,

DPPH radical scavenging %

$$= [(A_0 - A_1)/A_0] \times 100$$

Where A_0 is the absorbance of the DPPH solution and A_1 is the absorbance of the sample (Barros *et al.*, 2007).

Hydrogen peroxide radical inhibition assay (H_2O_2)

Ruch *et al.* (1989) were used the method to determine the H_2O_2 scavenging ability of extracts. The H_2O_2 scavenging capacities of the extracts were calculated using formula, (Ruch *et al.*, 1989).

H_2O_2 radical scavenging %

$$= [(A_{Blank} - A_{Sample})/A_{Blank}] \times 100$$

Reducing power assay

The extracts were prepared in different concentrations. Phosphate buffer (2.5 ml, 2 M, pH 6.6) and potassium ferricyanide were mixed with 1 ml of each in distilled water. The blend was incubated at 50°C for 20 min. A portion of trichloroacetic acid (TCA) was added to the combination which was then centrifuged at 2000 RPM for 20 min.

The superior layer of the solution was mixed with water and $FeCl_3$ (0.5ml), and the absorbance was calculated at 700 nm. Increased reducing power was indicated by increased in absorbance of the reaction mixture (Yıldırım *et al.*, 2001).

RESULTS AND DISCUSSION

Pharmacognostical studies

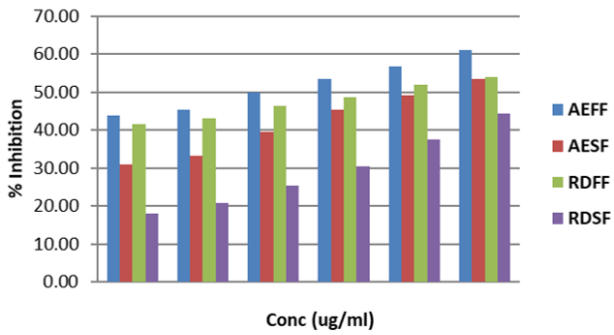
Physical Evaluation

The correct identity of the leaves of *Ailanthus excelsa* and the fruits *of Randia Dumetorum* is determined by pharmacological research. Morphological research of drugs includes evaluation of drugs by colour, odour, taste, size, shape and unique features, like touch, texture etc.

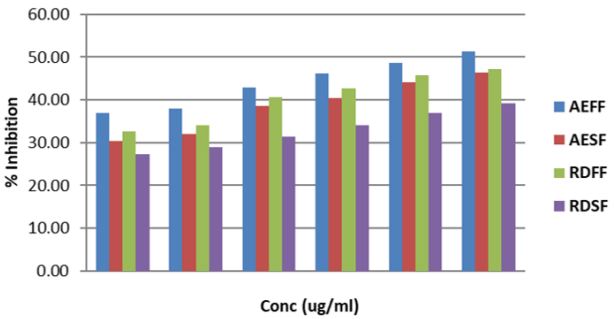
It evaluates special features, among others. It is a qualitative assessment technique based on an analysis of the morphological and sensory profiles of each drug (Table 2).

Physicochemical Evaluation

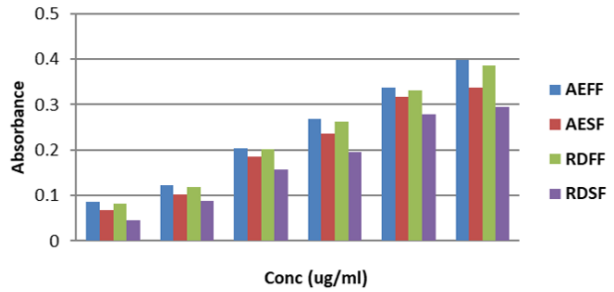
When raw materials are burned, ash residues are formed consisting of inorganic materials (metal salts and silica). This value is within a reasonably wide range and is, therefore, an essential parameter for evaluating raw materials. The value of the ash identifies more direct dirt, such as sand or dirt (Table 3).



Graph 1: Effect of Fractions in DPPH free radical scavenging assay



Graph 2: Effect of Fractions in Hydrogen peroxide scavenging assay



Graph 3: Effect of Fractions in Hydrogen peroxide scavenging assay

Phytochemical Investigations

Phytochemical screening of crude extracts of leaves of *A.excelsa* and fruit of *R. Dumetorum* by using chemical method revealed the presence of bioactive constituents such as Alkaloids, Carbohydrates, Flavonoids, Saponins, Tannins and Phenolic compounds, Glycosides (Table 4 and Table 5).

TLC characterisation

TLC is primary; easy to use an analytical method, and the solvent used are not hazardous and do not require advanced tools. Depending on the solvent fraction compounds, the solvent system was chosen. TLC examined all fragments of *A. excelsa* and *R. Dumetorum*, and the results of this study

Table 1: Concentration of different extracts for Antioxidant activity

AEFF (μg/ml)	AESF (μg/ml)	RDFF (μg/ml)	RDSF (μg/ml)
10	100	10	100
20	200	20	200
40	400	40	400
60	600	60	600
80	800	80	800
100	1000	100	1000

Table 2: Morphological characters

Sr. No.	Particulars	*Ailanthus excelsa* Leaves	*Randia Dumetorum* Fruit
1.	Colour	Greyish green	yellowish-brown
2.	Odour	Pleasant	Unpleasant
3.	Taste	Slightly Bitter	Bitter
4.	Length	40–100 cm	1.8 – 4.5 cm
5.	Surface	soft and velvety	Smooth
6.	Shape	Pinnate	Ovoid

Table 3: Determination of Ash values of crude drugs

Sr. No.	Particulars	*A. excelsa* Leaf (% w/w)	*R. Dumetorum* Fruit (% w/ w)
1.	Total ash	9.00	10.50
2.	Acid-insoluble ash	1.92	0.5
3.	Water-insoluble ash	6.10	4.65

are, flavonoids and saponins are present infractions (Table 6).

Yield Percentage, Total Phenolics and Total Flavonoids

The fraction yield percentage, total phenolics and flavonoid contents of the extracts obtained from the aqueous extract of *A.excelsa* leaves and fruit of *R. Dumetorum* are presented in Table 7. Among the different fractions, the maximum yield was obtained for the Flavonoid fraction of *R. Dumetorum* (4%) followed by *A.excelsa* (3%). For total flavonoid content (TFC) estimation standard curve of Rutin was used, and content was estimated as Rutin equivalent (RE). For total phenol content estimation (TPC) standard curve of Gallic acid was used and estimated as Gallic acid equivalent (GAE). TFC in *Ailanthus excelsa* and *Randia Dumetorum* were found to be 26 and 19 μg/mg RE respectively. Total Phenol Content in *Ailanthus excelsa* and *Randia Dumetorum* were found to be 65.14 and 62.44 μg/mg GAE, respectively.

Studies of Antioxidant Activities

Due to the complexity of phytochemicals, the antioxidant activity of plant extracts cannot be evaluated using a single method. Therefore, it is vital to use generally accepted tests to evaluate the antioxidant activity of plant extracts. Many methods of antioxidants have been developed to evaluate antioxidant activity and explain how antioxidants work.

DPPH free radical scavenging activity

The DPPH oxidation test used in this document to measure the capacity of radical-scavenging capacity (RSC) is used worldwide. The ability of radical biological agents to remove DPPH can be expressed as a unit capable of producing antioxidants. The DPPH alcohol solution has a bright purple colour, with an absorption peak at 517 nm when one of the radical scavengers disappears in the reaction system and only one electron of nitrogen is attached to the DPPH. The reaction rate and potential of the radical promoter depend on the rate and maximum value of the DPPH event (Sharma and Gupta, 2008; Gupta and Sharma, 2010).

Compared to other methods, the DPPH test has many advantages, such as good stability, reliable accuracy, simplicity, and feasibility. The results of the DPPH process are presented in several ways. Most studies indicate an IC50 value that is defined as the amount of antioxidant needed to reduce the initial DPPH concentration by 50.

Table 4: Preliminary Phytochemical testing of parent Ethanolic extract

Sr. No	Test	Observation	A. excelsa Leaf	R. Dumetorum Fruit
A. Alkaloids				
1	Hager's test	Yellow ppt	(+)	(+)
2	Mayer's test	White ppt	(+)	(+)
3	Wagner's test	Reddish brown ppt	(+)	(+)
B. Carbohydrates				
1	Molish's Test	Violet ring formed	(+)	(+)
2	Fehling's test	Brick red ppt	(-)	(+)
3	Barfoed's test	No change	(-)	(-)
C. Cardiac glycosides				
1	Legal test	No red color	(-)	(-)
2	Keller Killiani test	No change	(-)	(+)
D. Flavonoids				
1	Shinoda test	Pink color	(+)	(+)
2	Lead acetate test	Yellow color	(+)	(+)
E.Protein and amino acids				
1	Biuret test	No change	(-)	(+)
2	Millon's test	No change	(+)	(-)
3	Ninhydrin test	No change	(+)	(-)
F.Saponins				
1	Foam test	No foam formation	(+)	(+)
G.Steroids				
1	Salkowski reaction	Yellow fluorescence	(+)	(-)
2	Liebermann – Burchard reaction	Green color	(+)	(+)
H.Tannins and Phenolic compounds				
1	Ferric chloride test	Deep blue-black color	(+)	(-)
2	Gelatin test	White ppt	(-)	(+)
3	Lead acetate test	White ppt	(+)	(+)
4	Potassium dichromate test	Red ppt	(+)	(+)
5	Acetic acid test	Red color	(+)	(-)
6	Iodine test	Red color	(+)	(+)
I.Glycosides				
1	Borntrager's Test	NA	(+)	(+)
J.Mucilage				
1	Ruthenium red	NA	(-)	(+)
K.Cyanogenetic glycoside				
1	Sodium picrate test	NA	(-)	(-)

Table 5: Chemical test for rich fractions

Sr.no.	Test	AAFF	RDFF	Inference
Flavonoid rich fraction of crude drugs				
1.	Shinoda test	+	+	Flavonoids present
2.	Acid base test	+	+	Flavonoids present
Saponin rich fraction of crude drugs				
Sr.no.	**Test**	**AASF**	**RDSF**	**Inference**
1.	Foam test	+	+	Saponins present

Table 6: TLC characterization of Fractions

Sr. No	Mobile Phase	Spraying Reagent	No. of Spots		Rf Value		Inference
			AEFF	RDFF	AEFF	RDFF	
1	Chloroform: Methanol	Ammonia vapour/ VS reagent	2	1	0.25, 0.80	0.65	Flavonoids
2	Ethyl acetate: Formic acid: Glacial acetic acid: Water	Ammonia vapour/ VS reagent	2	3	0.34, 0.50	0.25, 0.45, 0.75	Flavonoids
			AESF	**RDSF**	**AESF**	**RDSF**	
1	Chloroform: Gallic acid: Methanol: Water	Anisaldehyde-sulphuric acid reagent	1	2	0.25	0.45, 0.70	Saponins
2	Chloroform: Methanol: Water	Vanillin-phosphoric acid reagent	2	2	0.15, 0.19	0.55, 0.60	Saponins

Table 7: Extract yield percentage, total phenolics and total flavonoid content in *A.excelsa* and *R. Dumetorum*

S. No.	Extract	% Yield	Total Phenolic Content	Total flavonoid content
1.	AEFF	3	65.14	26
2.	AESF	2.3	NA	NA
3.	RDFF	4	62.44	19
4	RDSF	2.6	NA	NA

Table 8: Effect of Fractions in DPPH free radical scavenging assay (% Inhibition)

S. No	Conc. (ug/ml)	AEFF	AESF	RDFF	RDSF
1	10	43.91	30.99	41.51	17.89
2	20	45.38	33.21	43.17	20.84
3	40	49.81	39.66	46.49	25.46
4	60	53.50	45.38	48.71	30.44
5	80	56.82	49.07	51.85	37.45
6	100	61.07	53.50	54.06	44.28
	IC_{50}	42.63	83.42	68.92	123.22

Table 9: Effect of Fractions in Hydrogen peroxide scavenging assay (% Inhibition)

S. No	Conc. (ug/ml)	AEFF	AESF	RDFF	RDSF
1	10	36.95	30.40	32.51	27.24
2	20	38.00	31.90	34.01	28.97
3	40	42.89	38.53	40.63	31.30
4	60	46.12	40.33	42.74	34.01
5	80	48.68	44.02	45.67	37.02
6	100	51.32	46.28	47.18	39.13
	IC_{50}	88.47	115.75	108.4	181.13

Table 10: Effect of Fractions in Hydrogen peroxide scavenging assay (Absorbance)

S. No	Conc. (ug/ml)	AEFF	AESF	RDFF	RDSF
1	10	0.087	0.068	0.082	0.046
2	20	0.122	0.103	0.118	0.088
3	40	0.204	0.186	0.201	0.157
4	60	0.269	0.236	0.263	0.195
5	80	0.337	0.317	0.332	0.278
6	100	0.398	0.338	0.386	0.294

This value is measured by plotting inhibition proportion against Fractions concentration. However, for plant extracts or pure compounds, the IC_{50} Value changes according to the final concentration of the DPPH used. AEFF showed IC_{50} of 42.63 μg/ml, AESF showed IC_{50} of 83.42 μg/ml, RDFF showed IC_{50} of 68.92 μg/ml, and RDSF showed IC_{50} of 123.22 μg/ml against DPPH radical (Table 8 and Graph 1).

Hydrogen peroxide scavenging

Hydrogen peroxide itself is not very reactive, but it sometimes becomes toxic to cells because it can carry hydroxyl radicals into the cells. Therefore, the elimination of H_2O_2 is essential to protect antioxidants in cell or food systems. It was noted that all of the samples tested in this study had the effect of removing hydrogen peroxide (Pietta, 2000). IC50 values are 88.47, 115.75, 108.4, and 181.13 for AEFF, AESF, RDFF, and RDSF respectively (Table 9 and Graph 2)

Reducing power assay

All test samples were also found to be having better-reducing power with good goodness of fit (R^2 > 0.9) for all test samples. Substances with reduced potency react with potassium burkianide (Fe3 +) to form potassium burkianide (Fe2 +) and then react with iron chloride with the absorption of up to 700 nm to form a ferric, ferrous complex iron (Table 10 and Graph 3). With an increase in concentration, absorbance increased for all test samples (Wenli *et al.*, 2004).

CONCLUSION

Pharmacological standardisation of the two plants includes physicochemical assessment, identification, verification, and detection of adulteration and quality control of the crude drug. Hot aqueous extract from the leaves of *Ailanthus excelsa* and the fruits of *Randia Dumetorum*, which have the most significant value for extraction. Therefore, we come to the breakdown of the aqueous extract. Analysis of the ash value shows that inorganic substances are present in very normal quantities. If physical and chemical methods are not sufficient, a phytochemical test can be used identified from their impurities. Phytochemical tests are helpful to detect various phyto components. Over the past two decades, the TLC method has been an essential tool for qualitative and quantitative phytochemical analysis of herbal products and preparations. The results of this study show that the leaves of *Ailanthus excelsa* and the fruits of *Randia Dumetorum* showed free radical scavenging, Hydrogen peroxide scavenging and reducing power activity. The antioxidant activity of each fraction of the two plants can be attributed to the polyphenol content and phytochemical components. The results of this study suggest that fractions of both plants could be a natural source of antioxidants as a therapeutic agent for a biological system that is sensitive to free-reaction intermediates.

ACKNOWLEDGEMENT

The authors thank Rajarshi Shahu college of pharmacy, Buldana for providing facilities to conduct the research.

Conflict of Interest

There are no conflicts of interest among all the authors with the publication of the manuscript.

Funding Support

The authors declare that they have no funding support for this study.

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